COMMONWEALTH BUREAU OF HELMINTHOLOGY,
THE WHITE HOUSE,
103, ST. PETER'S STREET,
ST. ALBANS, HERTS.

NEMATOLOGICA

INTERNATIONAL JOURNAL OF NEMATOLOGICAL RESEARCH

VOLUME III

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LEIDEN
E. J. BRILL
1958

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PRINTED IN THE NETHERLANDS

J. H. Schuurmans Stekhoven
1892-1958

J. H. SCHUURMANS STEKHOVEN Jr.

obiit 11 November, 1958

It is with deep regret that we announce the sudden death of the Editor-in-Chief of our Journal, Professor Dr. J. H. Schuurmans Stekhoven Jr., who died of a heart-attack at his home on November 11th at the age of 66.

A remarkable man and a very enthusiastic biologist has gone; a man who worked with untiring zeal on the numerous large projects which he undertook, who never stopped seeking new paths and was always busy training himself as for example by learning new languages which would be useful in understanding the works of his colleagues.

The testimonies of his work are found in some 250 publications, including books, monographs, papers in scientific and popular journals, and in newspapers. At the time of his death he was engaged among other things on a new edition of his "Manual of Agricultural Helminthology", on a dictionary of six thousand pharmaceutical words in six languages, five thousand of which were ready for print. Thanks to his tenacious memory, it is possible to find in his publications facts and references which cannot be found elsewhere.

The scientific merits of Prof. Schuurmans Stekhoven were not confined to the field of nematology: he was mainly parasitologist, and most of his work was done on parasites of man and domestic animals. Space does not permit a fuller consideration of these aspects of his work.

In the autumn of 1954 Schuurmans Stekhoven came to Wageningen with a proposal by the Publishers, E. J. Brill of Leiden, to start a new journal on the field of Nematology. With his usual enthusiasm he had convinced the publisher that a new journal on this field would have a real chance of success.

Starting a new journal is always a difficult undertaking, and this project did not meet with general approval. However, his tenacity overcame all objections. It was, moreover, a very happy coincidence that the biennial Symposium on

"Nematodes and the Diseases they Cause", was to take place in the next year, 1955, at Wageningen. Here, about 100 nematologists from about 20 countries gathered and the proposed journal met with unexpected approval from all sides, for in several countries nematologists were in need of more space in journals and, apparently, painstaking original work had to wait too long before it could be published. In that way the foundation of Nematologica was laid, and publication commenced in 1956 with the communications of the Symposium at Wageningen. Therefore, nematologists will remember the name of Schuurmans Stekhoven; it will be linked with this journal Nematologica which would not have existed but for his convincing personality.

Next to Nematologica, the name of Schuurmans Stekhoven will live as the co-author of the "Manual of Agricultural Helminthology", commenced by I. N. Filipjev and translated and finished by Schuurmans Stekhoven. The book is a mine of bibliographic data and descriptions of species, and is therefore indispensable to every nematologist. It is a great pity that his death has intervened before the performance of a new edition of this book, on which he was working ardently.

Born the son of a psychiatrist, he was already in his boyhood a keen lover of natural history, and his intention to become a biologist existed from his sixth year. In his study at the University of Utrecht, he turned to Zoology and his thesis "De sexualiteit der Myxosporidia" which gave him on 5 June, 1918 his doctor's degree (*cum laude*) showed evidence of work of high standard. After a short period on tropical, veterinary parasitology, done in Java, he returned to Utrecht and for a period of almost twenty years taught parasitology to the students of biology. A Rockefeller stipendium in 1926 enabled him to work at the Department of Agriculture, Washington. He crossed the atlantic a second time, to become a Professor in General Biology at the University of Tucuman, Argentina. From 1952 to 1958 he was the leader of the biological laboratories of the chemical firm of Noury & Van der Lande, Deventer, the Netherlands.

Editorial Board & Publishers
Nematologica

CONTENTS OF VOLUME III

ANDRÁSSY, I., *Hoplolaimus tylenchiformis* Daday, 1905 (Syn. *H. coronatus* Cobb, 1923) und die Gattungen der Unterfamilie Hoplolaiminae Filipjev, 1936 44

——, *Diplogaster lepidus* n.sp. und der Schlüssel der *Diplogaster*-Arten von unpaarigem Ovar 295

BIRD, A. F., The adult female cuticle and egg sac of the genus *Meloidogyne* Goeldi, 1887 205

BISHOP, DAPHNE, A technique for screening antibiotics against eelworms 143

BROWN, E. B. Pea root eelworm in the eastern counties of England . 257

CARROLL, K. K., Purification and properties of eelworm hatching factors 197

—, HEYES, J. K., JOHNSON, A. W. & TODD, A. R., The potato eelworm hatching factor 7. Further methods of concentration of the factor . 154

DROPKIN, V. H., MARTIN, G. C. & JOHNSON, R. W., Effect of osmotic concentration on hatching of some plant parasitic nematodes . . 115

ELLENBY, C., Day length and cyst formation in the potato root eelworm, *Heterodera rostochiensis* Wollenweber 81

GOFFART, H. & HEILING, A., Nebenwirkungen bei der Nematodenbekämpfung mit Shell D-D und verwandten Mitteln 213

GOODEY, J. B., *Paraphelenchus myceliophthorus* n.sp. (Nematoda: Aphelenchidae) 1

——, *Ditylenchus myceliophagus* n.sp. (Nematoda: Tylenchidae) . . . 91

——, *Sphaeronema minutissimum* n.sp. (Sphaeronematinae: Tylenchulidae) 169

—— & HOOPER, D. J., Observations of the effects of *Ditylenchus dipsaci* and *Anguina tritici* on certain wheat and barley varieties . . 24

GUNDY, S. D. VAN, The life history of the citrus nematode, *Tylenchulus semipenetrans* Cobb 283

HAGUE, H. G., The concentration of potato root diffusate under reduced pressure 149

HESLING, J. J., *Heterodera major* O. Schmidt, 1930. Population changes in the field and in pots of fallow soil 274

HOOPER, D. J., *Aphelenchoides dactylocercus* n.sp. and *A. sacchari* n.sp. (Nematoda: Aphelenchoidea) 229

LOOF, P. A. A., Some remarks on the status of the subfamily Dolichodorinae, with description of *Macrotrophurus arbusticola* n.g., n.sp. (Nematoda: Tylenchidae) 301

—— & OOSTENBRINK, M., Die Identität von *Tylenchus robustus* De Man 34

LUC, M., Trois nouvelles espèces Africaines du genre *Hemicycliophoru* De Man, 1921 (Nematoda: Criconematidae) 15

——, *Xiphinema* de l'Ouest Africain: Description de cinq nouvelles espèces (Nematoda: Dorylaimidae) 57

MARTIN, G. C., Root-knot nematodes (*Meloidogyne* spp.) in the Federation of Rhodesia and Nyasaland 333

OOSTENBRINK, M., An inoculation trial with *Pratylenchus penetrans* in potatoes 30

PAETZOLD, D., Beobachtungen zur Stachellosigkeit der Männchen von *Hemicycliophora typica* de Man, 1921 (Criconematidae) 140

PITCHER, R. S. & CROSSE, J. E., Studies in the relationship of eelworms and bacteria to certain plant diseases. II 244

SAUER, M. R., *Hoplolaimus gracilidens, Radopholus inaequalis,* and *Radopholus neosimilis,* three new tylenchs native to Victoria, Australia . 97
SHEPHERD, AUDREY M., Experimental methods in testing for resistance to beet eelworm, *Heterodera schachtii* Schmidt 127
SIMON, LUDWIG, Nematologische Untersuchungen an Hopfen. II. Zur Morphologie und Biologie von *Heterodera humuli* Filipjev, 1934 . . 269
THOMAS, P. R., Severe eelworm (*Ditylenchus dipsaci* (Kühn) Filipjev) infestation of the narcissus variety Soleil d'Or 73
TRACEY, M. V., Cellulase and chitinase in plant nematodes . . . 179
WALLACE, H. R., Observations on the emergence from cysts and the orientation of larvae of three species of the genus *Heterodera* in the presence of host plant roots 236
WEERDT, L. G. VAN, Studies on the biology of *Radopholus similis* (Cobb, 1893) Thorne, 1949. II 184
WHITEHEAD, A. G., *Rotylenchoides brevis* n.g., n.sp. (Rotylenchoidinae n. subfam.: Tylenchida) 328
WIDDOWSON, E., Potato root diffusate production 6
——, The production of root diffusate by potatoes grown in water culture 108
——, Observations on the collection and storage of potato root diffusate 173
——, DONCASTER, C. C. & FENWICK, D. W., Observations on the development of *Heterodera rostochiensis* Woll. in sterile root cultures . 308
WINNER, CHR., Untersuchungen über die Eigenschaften der auf *Heterodera schachtii* Schmidt aktivierend wirkenden Wurzelexsudate von *Brassica rapa oleifera* D.C. 315
WINSLOW, R. D., The taxonomic position of *Anguillulina obtusa* Goodey, 1932 and 1940 136

Book reviews: I. ANDRÁSSY, Szabadonéló fonálférgek Nematoda libera, by Á. Soós 350
C. L. DUDDINGTON, The friendly Fungi, by P. A. van der Laan . . 168
Scientific Notes: H. GISIN, Formation of an international group for the study of the fauna of the soil 79
A. C. TARJAN, Note on the selection of types for certain nematode species 79
A. C. TARJAN, A checklist of soil and plant-parasitic nematode species 80
Society of European Nematologists, Fifth International Nematology Symposium 351
Errata: J. DIJKSTRA; H. GOFFART & A. HEILING 5, 314

Nematologica III (1958) : 1-5. E. J. Brill, Leiden

PARAPHELENCHUS MYCELIOPHTHORUS N.SP. (NEMATODA: APHELENCHIDAE)

BY

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During investigations into the relationships of mushrooms and various nematodes, a new species of *Paraphelenchus* was encountered which was capable of destroying the mycelium. It is hereby named *Paraphelenchus myceliophthorus* n.sp., the specific epithet indicating its behaviour as a destroyer of mycelium, and a description follows.

Measurements were made on specimens fixed in TAF (Courtney, Polley & Miller, 1955) and then processed by the rapid method and mounted in glycerine (Goodey, 1957). Observations were made on living and preserved specimens.

Paraphelenchus myceliophthorus n.sp.

50 ♀♀ L = 0.699 mm (s = ± 0.058 mm) (measured range 0.58-0.82 mm)
a = 22-34; b = 4.1-6.6; c = 13-24; V = 26-55 71-78 9-15
50 ♂♂ L = 0.691 mm (s = ± 0.060 mm) (measured range 0.56-0.82 mm)
a = 23-32; b = 3.9-5.9; c = 14-30; T = 46-47

Holotype ♀ L = 0.79 mm; a = 26; b = 4.8; c = 18; V = 36 75 10.6
Allotype ♂ L = 0.79 mm; a = 24; b = 5.6; c = 22; T = 64

Fine transverse striations, about 0.8 μ apart, occur on both cuticle and sub-cuticle. These striae are interrupted on either side of the body by the lateral fields which occupy about a third of the body-width and which are marked by six incisures. Anteriorly these incisures reduce, on the female, from six to five over the fore-part of the intestine, from five to four at the base of the oesophagus, from four to three between the corpus and spear base, and finish as two at approximately the spear base. Posteriorly there are six incisures at the vulva, these have reduced to four at the end of the post-vulval sac and they terminate near the tail tip as four. In some specimens the lateral field widens out on the tail or at the level of the vulva but the number of incisures remains unaltered. In the male the general pattern of the

incisures is similar but on the tail the four splay out a little as they end towards the ventral side.

The head is not offset from the body and its cuticle is devoid of striae (Fig 1e). An end-on view shows the six lips, slightly flattened in the dorso-ventral plane, but neither the openings of the amphids nor any head papillae are discernible. The only sclerotization in the head consists of six short circum-oral rods fused to form a stellate circlet through which the spear is guided: there is no basal plate. The spear is about 16 μ long with a pointed fore-part about 5 μ long and a shaft about 11 μ long: it lacks basal knobs. The procorpus is long and cylindrical and the lumen of the oesophageal duct is relatively wide. The corpus is elliptical with a relatively large refractive valvular apparatus. The dorsal oesophageal gland opens in the anterior part of the corpus and the subventrals just behind the valvular apparatus. The glands are contained in a slightly spathulate basal bulb which is a little shorter than the procorpus and which joins the intestine without any overlap. The junction of the oesophagus and intestine is displaced towards the dorsal side of the body and in the space below is found a single elongate uninucleate cell which is joined to the excretory pore by a narrow duct. This duct appears to be in two parts of approximately equal length, that part proximal to the cell being more highly refractive than the other. The excretory pore opens about a half body-width behind the corpus and is four or five annules anterior to the hemizonid. The latter extends over three or four annules and is easily seen in fixed specimens but is rather obscure in live ones. The nerve ring encircles the isthmus just behind the corpus and prominent deirids are found in the lateral fields at the level of the hemizonid. In the female the intestine ends in a rectum which may be as much as twice as long as the anal body-width. The rectum is slightly flattened in the dorso-ventral plane and the anus is a transverse slit (Fig 1b, 1f). The tail of the female ends in a pair of short papillae (Fig 1b) which each have a central core connected with the body contents. The pre-adult larva has a short ventral mucron on the end of the tail.

The female has a single prodelphic gonad with a post-vulval sac extending two-thirds of the distance from vulva to anus (Fig. 1f). The outstretched ovary seems to be composed of a number of oocytes in one or more rows but only the nuclei are visible and not the division into separate cells. At the base of the ovary there are about eighteen closely packed cells surrounding the oviduct and these connect the

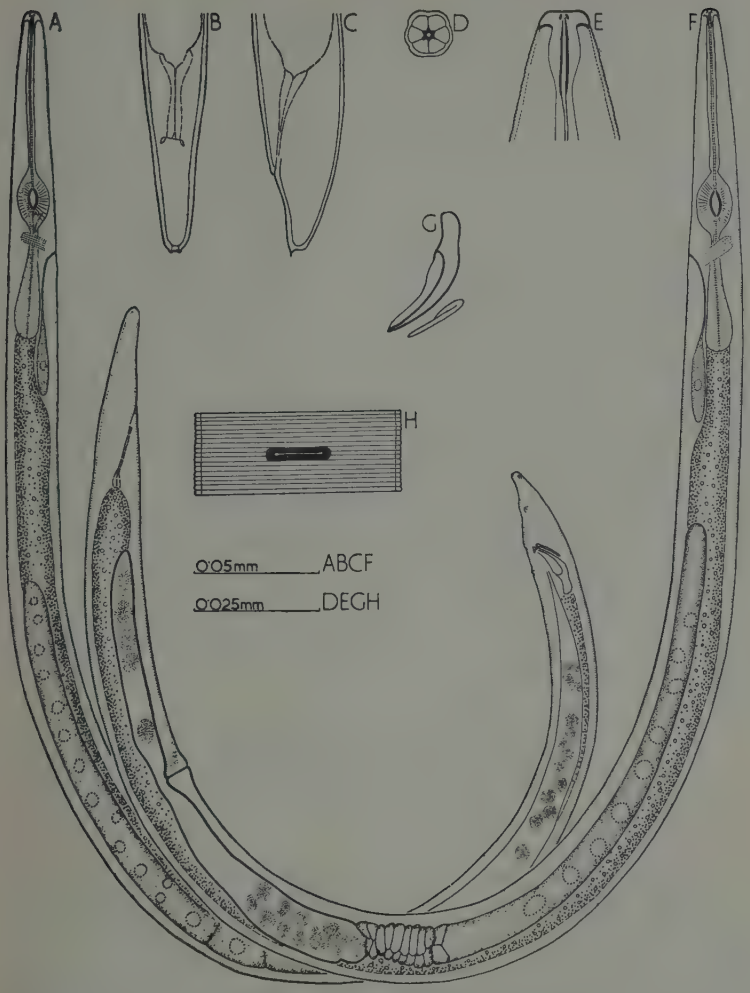

Fig. 1. A ♂ nematode; B ♀ tail in ventral view; C larval tail in lateral view, D end-on view of head; E lateral view of head end; F ♀ nematode; G spicule and gubernaculum in lateral view; H vulva in ventral view.

ovary to the wide uterus. The cavity of the uterus and that of the post-vulval sac contain sperms. No gravid specimens were seen but eggs were found in the medium when the nematodes were cultured on agar plates of mushroom; these measured about 78 μ by 32 μ. The uterus

connects with the vulva, which is a transverse slit about one third of a body-width long, through a short vagina which has muscular walls and is set at right angles to the body wall.

In the male there is a single gonad. The outstretched testis, like the ovary, shows practically no division into cells, though its nuclei are fairly easily discernible. The two spicules are slightly curved and about 28 μ long; the gubernaculum about 13.5 μ long. The blunt tail has no bursa but bears a number of papillae: one pair sub-ventral and adanal, one pair sub-ventral half way down the tail, and two pairs at the tail tip, one sub-dorsal and the other sub-ventral. There is also a single median ventral papilla slightly pre-anal in position; in some cases this is absent and in others there is a pair of sub-ventral papillae in this position (Fig. 1a).

Differentiation. There are three other species of *Paraphelenchus* described: *P. pseudoparictinus* (Micoletzky, 1922) Micoletzky, 1925, *P. amblyurus* Steiner, 1934 and *P. arenaceus* T. Goodey, 1944 syn. *Aphelenchus agricola* de Man, 1921. *P. myceliophthorus* differs from all these in the pattern exhibited by the papillae of the male tail, though it is fairly similar to *P. amblyurus* where, however, there is a mucron on the tail. The female tail is also different in the absence of a mucron; one is present in *P. pseudoparietinus* and one or more in *P. amblyurus*.

Type host. Mycelium of *Agaricus hortensis* Cooke, the cultivated mushroom.

Type habitat. Mushroom compost, West Chiltington, Sussex.

Type slides. Holotype ♀ slide no. 88/3/1, Allotype ♂ slide no. 88/3/2 in the collection of the Nematology Department, Rothamsted Experimental Station.

Two points call for comment. 1. The pair of papillae on the female tail are very similar to those figured by Steiner (1943) for *Metaphelenchus rhopalocerus* Steiner, 1943, which he indicates are the phasmids displaced almost to the tail tip. 2. In *P. myceliophthorus* the excretory duct can be traced, in live specimens, from the excretory pore to the uninucleate cell lying ventral to the junction of oesophagus and intestine. Steiner (1934), describing *P. amblyurus says* "the renette or ventral gland seems to consist of one large and two minute cells located ventrad of the esophageal bulb", and he figures this structure but does not show the connection between it and the excretory pore. In the files of the late T. Goodey a drawing of *P. pseudoparie-*

tinus also shows a ventral cell with the suggestion of a duct connecting it with the excretory pore. It seems, therefore, that the excretory system of *Paraphelenchus* does, in fact, consist of a ventral cell connected by the excretory duct to the excretory pore.

ZUSAMMENFASSUNG

Paraphelenchus myceliophthorus n.sp. wird beschrieben und abgebildet. Der Nematode greift das Mycel der in Kompost wachsenden Pilze an. Der Excretionsgang verbindet den Porus mit einer ventralen uninuclearen Zelle. *P. myceliophthorus* unterscheidet sich von anderen Arten durch die Form der Papille am Schwanz des Männchens und durch das Fehlen eines Mucrons am Schwanz des Weibchens.

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(*Received for publication*: *June 4, 1957*)

ERRATUM

Nematologica II (J. DIJKSTRA).

Page 230, line 2 and 4 under Table I, and Plate XIX in the caption of fig 3, instead of above and below *read* left and right respectively. Page 234 footnote, to row figures one more figure 4.5 should be added.

Nematologica III (1958): 6-14. E. J. Brill, Leiden.

POTATO ROOT DIFFUSATE PRODUCTION

BY

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Pot tests were made to assess the effects of plant age, variety, and an infestation of potato-root eelworm, *Heterodera rostochiensis* Woll., on the activity of root diffusate produced by potted potatoes; the diffusates produced by one variety of potato and one variety of tomato were also compared.

Technique

The same methods for growing the plants, leaching out, and assaying the diffusates, were followed in all the pot tests. Chitted tubers of the variety Arran Banner were used throughout. Each was approximately 2 oz. in weight and all the shoots but one were cut out of each tuber before they were planted in coarse horticultural sand in 6½-in. diameter pots. The plants were fed with a nutrient solution, twice weekly and grown in a cool glasshouse during the early summer. The leaching was started when the potato shoots were just showing through the sand, at "breakthrough", this was usually about two weeks after planting. Pot replication in each test was three fold except in the potato and tomato series where five replicates were used.

Twenty-four hours before leaching, the selected pots were washed through to remove any diffusate and nutrients present. To avoid extremes of temperature they were left overnight in a cool soil room, at an average root temperature of 12°-14° C. The diffusate was leached out with 200 ml. of tap water which was allowed to drain through the pot completely, made up to 250 ml. and then put through a further four times. After leaching, the roots were washed, the surface moisture taken off on an absorbent cloth and the roots and shoots weighed separately. The leachates were stored at +2° C until the end of each pot series when they were assayed against an active standard diffusate at several dilutions using the methods of FENWICK & WIDDOWSON (in press). Batches of 100 cysts were set up in five or ten fold replication

per sample of leachate. Larval emergence in the undiluted standard diffusates reached approximately 85 % of the total cyst contents in the cyst stock used. Regression lines giving calculated L.A. values [1]) (FENWICK, 1952), were computed for the hatch from the standard diffusate dilutions for each experiment and activities were usually about L.A. 3.0; from these, the L.A. values were derived for the leachate samples. Analyses of variance were made on the larval counts converted to logarithms and critical differences were derived. In the tables and graphs, mean L.A. values and root and shoot weights are used, based on three plant replicates.

The effect of age.

In the diffusate and plant age series, thirty pots were involved, three being leached at breakthrough, at 3 days, and at 1, 2, 3, 4, 6, 8 and 10

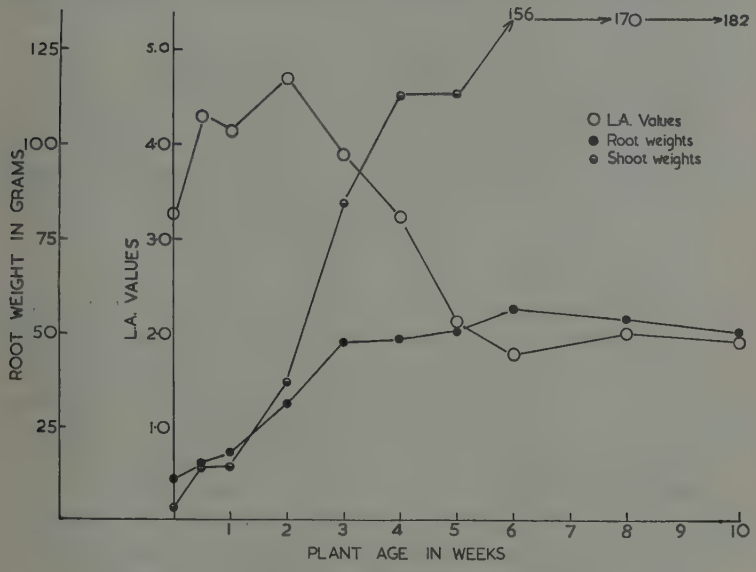

Fig. 1. The effect of plant age on diffusate production.

weeks after breakthrough. The hatching test involved 190 batches of 100 cysts with a standard diffusate at three dilutions, 1 : 1, 1 : 16 and

1) An L. A. value is the logarithm of the concentration of the active factor (expressed in arbitrary units) in any given sample of diffusate and is proportional to its activity.

1 : 256. The L.A. values and root and shoot weights are shown in Fig. 1.

The plants were producing an active diffusate by the time the shoots had broken through, production rose to a well defined peak two to three weeks later and began to decline four weeks after breakthrough. The time of maximum diffusate production coincided with a period of very rapid root growth when root weights rose from 11.5 g. to 49 g. per root in four weeks. The maximum root weight, 56 g., was reached by six weeks after breakthrough. Shoot weights also increased rapidly after the first week but this continued throughout the period of observation.

When this test was repeated the following season, slower growth was reflected in the activities of the leachates which did not show such a clearly defined peak nor reach such a high level of activity. The initial root weight was 7.3 g. which rose to 40 g. per root compared with 11.5 g. and 56 g. respectively in Experiment 1. Diffusate production was slow at first but reached a value of L.A. 3.0 one week after breakthrough and this level was maintained for six weeks.

The experiments showed that the most active diffusate is produced by young potato plants during the period of very rapid growth immediately after the shoots have shown through. Thus where potatoes are grown for continual diffusate collection, leaching can be started within a week after breakthrough and continued for about six weeks.

The effect of variety

In the variety test four early, three second early and three main crop potato varieties were grown and leached at 1, 3 and 6 weeks after breakthrough. This resulted in 90 samples of diffusate which were tested against a sample of stock diffusate of L.A. 3.7 at four dilutions. The varieties and the activity values of the leachates from them are listed in Table I.

None of the samples were of high activity possibly due to the slow and rather uneven rate of growth of all the plants. The early types had made the most growth on average up to the first leaching but this was not reflected in the activity of the leachates, the highest value L.A. 2.4 being from Majestic with a root weight of 7.8 g. compared with 7.8, 14.4, 13.9, 15.3 for the earlies. The effect of time on leachate activity was significant at the $p = 0.05$ level of significance but the interaction of variety with time was not. The diffusate from the Majestic potatoes was the most active, giving a larval hatch which was significantly greater than that from the Epicure, Duke of York or

TABLE I

L.A. values for ten varieties of potato at three ages. Expt. 1.

Variety	*Type*	*L.A. Values*		
		1 week	*3 weeks*	*6 weeks*
A. Epicure	Early	0.7	2.2	—
B. Arran Pilot	Early	1.4	1.9	1.5
C. Eclipse	Early	1.4	1.9	2.0
D. Duke of York	Early	0.8	1.5	2.5
E. Great Scot	Second early	1.1	1.9	1.9
F. Gladstone	Second early	2.1	1.6	1.9
G. Arran Banner	Second early	1.3	1.9	1.8
H. King Edward	Main crop	0.5	1.9	1.8
I. Ulster Chieftain	Main crop	1.7	1.8	1.2
J. Majestic	Main crop	2.4	1.9	1.8

King Edward varieties. King Edward was found to be significantly less active than all the rest except Duke of York. No real differences were found between the other varieties, the order of activity being, J, C, F, G, B, I, E, A, D, H.

When this experiment was set up again the result was roughly the same from plants which grew much more vigorously and produced diffusates of a higher activity. The varieties in order of activity were J, C, E, G, I, B, A, F, D, H, with the varieties Majestic and Eclipse again giving total hatches significantly higher than those from the Duke of York and King Edward potatoes.

In the first test the most active diffusates at breakthrough came from three varieties, Ulster Chieftain, Majestic and Gladstone. In the second, Ulster Chieftain, Eclipse and Majestic gave the highest activities at this stage. Apart from low hatches from Ulster Chieftain and Arran Pilot diffusates there was little difference in the first experiment between the activities of the other varieties after six weeks; Eclipse and Duke of York gave the highest result. Differences were more marked at six weeks in the second test, when the varieties Eclipse, Great Scot, Gladstone, Arran Banner and Majestic gave diffusates of very high activity; Epicure, Arran Pilot and King Edward gave the lowest values.

The most suitable varieties for diffusate production would appear to be Eclipse and Majestic since they gave active diffusate at breakthrough and up to six weeks after breakthrough, in each experiment.

The effect of infestation with potato-root eelworm

In the infestation experiments potatoes were grown in pots of sand inoculated with eelworm cysts to give levels of 102, 45 and 21 eggs

per gram and three pots of each were leached at breakthrough, and at 3, 6 and 9 weeks after breakthrough. Including a control series 48 pots were involved. The hatching test was set up against a standard diffusate series of L.A. 3.0 and a graph showing the L. A. values for each level of inoculum is given in Fig. 2, parts (a) and (b), together with the corresponding root weights. Apparently the plants at the medium population level were stimulated to increased diffusate production since throughout the experiment they gave the highest activities

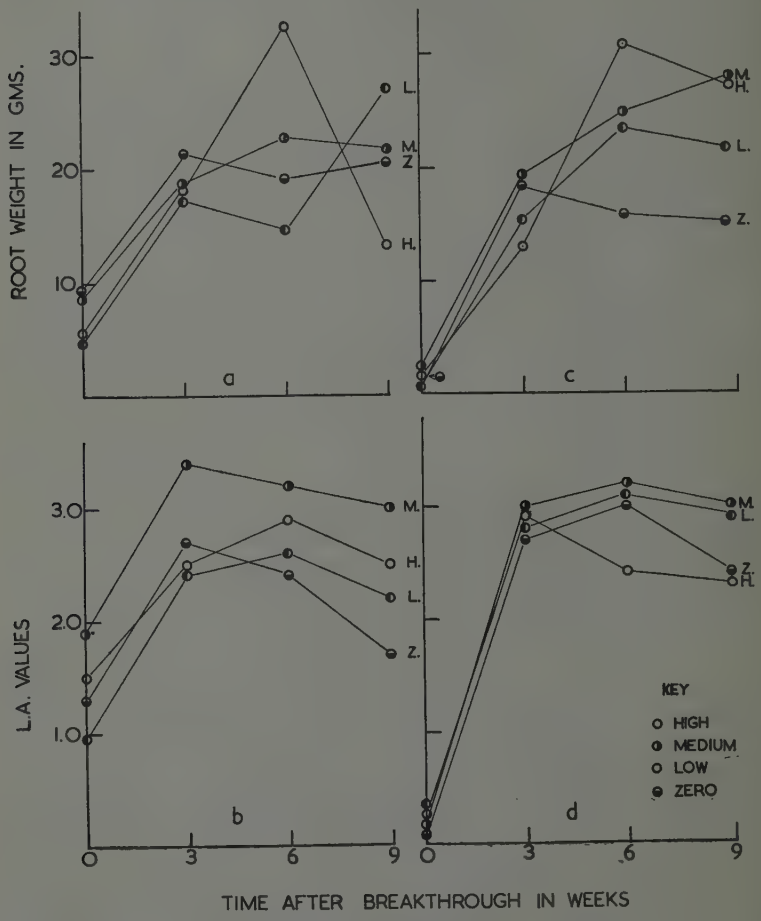

Fig. 2. The effect of infestation on diffusate production.

from weights of root not significantly different from those of the controls. The very marked increase in root weight in response to the heavy infestation was accompanied by only a slight increase in diffusate production, during the last stages the root weights fell, both effects probably resulting from injury due to a heavy infestation.

The analysis showed that the total larval emergence in the diffusates from the medium inoculation of 45 eggs per g. was significantly higher than that from the low and the zero levels. No difference was found between the total hatches in the diffusates from all the plants at three, six or nine weeks after breakthrough but in all cases the hatch from the first leachings was significantly lower than the rest.

In a repetition, the plants in the medium and in the low levels of inoculum showed an increased rate of diffusate production and again the heaviest level resulted in the biggest root weight up to six weeks after breakthrough without any corresponding increase in diffusate activity, in fact there was a notable decline from three weeks in the second test. The results of this experiment are shown in Fig. 2, parts (c) and (d). Analysis of the larval counts showed the total hatch from the medium inoculum level diffusate to be higher than that from the high or zero levels between which there was no difference.

In both experiments the potatoes grown in sand inoculated with 45 eggs and larvae per g. were producing the most active diffusate by the time the shoots showed through. This high level of production was clearly maintained for nine weeks, and in the second experiment was only exceeded by the diffusates from the light inoculation after nine weeks. In the first experiment the initial increase in root weight for six weeks after breakthrough at 120 eggs per g. was followed by a decline due to root damage, an effect less marked in the repetition.

Potato versus Tomato test

Tomatoes are known to give an active root diffusate suitable for hatching potato root eelworm larvae and are a more suitable crop for glasshouse culture than are potatoes. The activity of the diffusate from the two crops grown under the same conditions was therefore tested.

The tomato seeds were sown in pans of sand and when at the second rough leaf stage were transplanted singly to 6½-in. diameter pots of sand which had previously been saturated with nutrient solution and allowed to drain thoroughly. The potatoes were planted at the same time and leaching of both species started when the potatoes showed through the sand about twelve days later. The tomatoes were

then three to four inches high and about six weeks old. Plant replication in this experiment was five fold and leaching was done at breakthrough and at 1, 2, 3, 4, 6 and 9 weeks afterwards. The seventy diffusate samples were set up against seven dilutions of a standard diffusate of L.A. 3.1. The L.A. values and root weights of both species are given in Fig. 3.

The potatoes produced an active diffusate from one to six weeks after breakthrough with a peak at four weeks. There was a slow decline in activity after this in spite of a rising root weight, a condition observed in the previous experiments. The critical difference for sub-

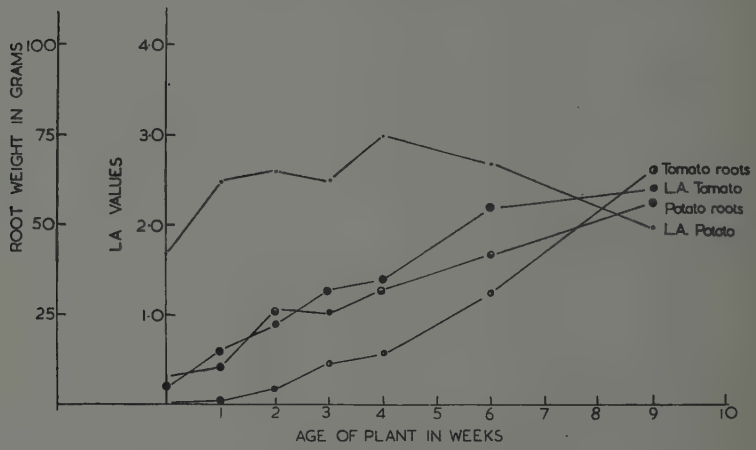

Fig. 3. Comparing the diffusate production of potato and tomato.

treatment totals showed that the first leaching was, and the last leaching was almost, significantly less active than the four week product.

Diffusate production by the tomatoes was the slower throughout the period of observation, under the conditions of the experiment. It is probable that had they been allowed a longer period for root establishment after transplanting diffusate of higher activity might have resulted. The potato diffusate reached a peak value of L.A. 3.0 four weeks after emergence where the root weight was 32 g. It was not until six weeks after transplanting, that the tomatoes achieved a root weight of approximately 31 g., the corresponding L.A. value was then L.A. 2.4. Statistical analysis showed that there was no difference between the larval emergence in the two types of diffusate at six and at nine weeks when the root weights were 31.4 and 65.0 for tomato and

40.7 and 56.4 for potato respectively, but the peak of potato diffusate production was then over. Since the intensive tomato root growth between six and nine weeks resulted in an increase of only 0.4 in L.A. value it was felt that the potatoes were the more efficient crop to use for the collection of root diffusate.

DISCUSSION

As a result of leaching potatoes of varying ages and variety, it was found that a peak of root diffusate production associated with intensive root growth was reached about four weeks after the emergence of the shoot. Diffusate from plants more than six weeks old was unreliable.

Although active diffusate was obtained from the ten potato varieties tested, Eclipse and Majestic were found to be the most satisfactory while King Edward and Duke of York gave a consistently low result.

Root growth and diffusate production were increased by growing potatoes in eelworm populations of 21 and 45 eggs and larvae per g., this was also noted by DONCASTER (1957). A heavier inoculum only slightly affected the output of diffusate but caused considerable root damage after six weeks in the first experiment.

A short term comparison between potato and an outdoor variety of tomato showed that the potatoes produced more diffusate per unit weight of root than tomatoes up to nine weeks after emergence, under cool glasshouse conditions.

ZUSAMMENFASSUNG

Topfversuche werden beschrieben, in denen der Einfluss von Alter, Sorte und Nematodenbefall auf die Wurzeldiffusatproduktion von Kartoffeln untersucht wurde. Die Pflanzen wurden in 15 cm grosse mit grobem Sand gefüllte Töpfe angezogen und mit 250 ml Wasser bewässert. Die Diffusate wurden im Schlüpfversuch mit je 100 Cysten in 5-facher Wiederholung geprüft.

Das am stärksten aktiv wirkende Diffusat bildete sich bis zu 4 Wochen nach dem Erscheinen der Triebe. Die Pflanzen füllten zu diesem Termin den Topf aus. Nach 6 Wochen nahm die Aktivität des Wurzeldiffusats ab.

Zehn im Handel befindliche Kartoffelsorten wurden geprüft und erbrachten Diffusate von annähernd gleicher Aktivität. Die Sorten „Eclipse" und „Majestic" zeigten in zwei Jahren eine bessere, „Duke of York" und „King Edward" eine geringere Aktivität ihres Wurzeldiffusats.

Wenn die Kartoffeln in Sand heranwachsen, der mit 21, 45 und 102 Eiern und Larven je g Boden infiziert war, wurde eine erhöhte Diffusatmenge aus den Pflanzen erhalten im Vergleich zu den Kontrollpflanzen. Obwohl die stärkste Population das kräftigste Wurzelwachstum bis zu 6 Wochen nach dem Erscheinen der Triebe in beiden Jahren hervorrief, kam es zu keinem entsprechenden Ansteigen in der Diffusat-Produktion, im zweiten Jahr war sie sogar vermindert.

Ein Vergleich zwischen Kartoffeln und Tomaten zeigte, dass Kartoffeln das meiste Diffusat je Wurzeleinheit bis zwei Wochen nach dem Erscheinen der Triebe produzierten. Es waren Anzeichen vorhanden, dass nach 9 Wochen das umgekehrte Bild eintrat.

ACKNOWLEDGEMENT

I wish to thank Dr. D. W. Fenwick for his advice during this work.

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TROIS NOUVELLES ESPÈCES AFRICAINES DU GENRE *HEMICYCLIOPHORA* DE MAN, 1921 (NEMATODA: CRICONEMATIDAE)

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L'intérêt porté aux Criconematidae et au genre *Hemicycliophora* De Man, 1921, en particulier s'est considérablement accru ces dernières années depuis la découverte, chez certaines espèces, d'un parasitisme actif envers le système souterrain des plantes cultivées. Nous renvoyons à TARJAN (1952) et surtout THORNE (1955), qui révisèrent ce genre, pour de plus amples détails sur le parasitisme des différentes espèces.

Au cours de recherches sur les nématodes phytoparasites poursuivies en 1955 et 1956 dans l'Ouest Africain Français, nous avons rencontré trois espèces du genre *Hemicycliophora*, espèces qu'il nous faut considérer toutes trois comme nouvelles.

La description de ces espèces porte à 5 le nombre d'espèces connues pour être bisexuées:

H. typica (De Man, 1921) Thorne, 1955
H. penetrans Thorne, 1955
H. membranifer Micoletzky, 1925 [1])
H. oostenbrinki n. sp.
H. paradoxa n. sp.

Chez la troisième espèce décrite, *H. pauciannulata* n. sp., nous

1) MICOLETZKY (1925) n'a pas vu le mâle de *H. membranifer*, mais LOOS (1948) a décrit sous le nom de *H. typica* de Man 1921, des individus appartenant très certainement à *H. membranifer*; en effet l'ornementation du champ latéral et des anneaux de la femelle correspond parfaitement au dessin original de MICOLETZKY et diffère notablement de celui donné par THORNE (1955) pour *H. typica*; de même le mâle présente un certain nombre de différences avec le mâle de *H. typica*: taille plus faible et champ latéral marqué de deux séries de blocs au lieu de deux simples lignes longitudinales. Il semble donc raisonnable de considérer les mâles et les femelles décrits par LOOS (1948) comme appartenant à *H. membranifer*. De même l'espèce donnée par MEYL (1955) comme *H. typica* est plus proche, par l'ornementation du champ latéral de la femelle, de *H. membranifer* que de *H. typica*.

n'avons pas rencontré de mâles, mais les femelles possèdent toutes une volumineuse spermathèque. Chez ces trois espèces les femelles et les mâles présentent un hémizonide très net. Cela confirme donc les vues de THORNE (1955) sur le rôle sexuel possible de cette structure qui ne se retrouve pas chez les femelles des espèces unisexuées sauf si elles possèdent une spermathèque (toutefois *H. tenuis* Thorne, 1955 espèce unisexuée sans spermathèque possède un hémizonide).

Hemicycliophora pauciannulata n. sp., fut trouvée au Togo en assez faible quantité au voisinage du système radiculaire de Cocotiers (*Cocos nucifera* L.) atteints de la „maladie de Kaincopé", dépérissement graduel se propageant rapidement au Togo et en Gold Coast et dont l'agent causal est encore inconnu. Si l'on ne retrouve pas cette espèce constamment associée aux cocotiers malades, on ne note jamais sa présence au voisinage de cocotiers sains. Les femelles de *H. pauciannulata* n. sp., comme le nom d'espèce l'exprime, ne sont pas toujours ornées de 200 anneaux, se situant donc, à ce point de vue, en dessous de la limite inférieure admise pour le genre *Hemicycliophora* et se rapprochant du maximum de 160 admis pour le genre *Criconemoides* Taylor, 1936.

H. oostenbrinki n. sp. est très fréquente en Côte d'Ivoire et en Guinée au voisinage de racines de bananiers (*Musa paradisiaca* L.), souvent en très grande quantité. Cette espèce est toujours associée à des *Tylenchidae*. Nous l'avons exceptionnellement rencontrée au voisinage de racines d'Ananas (*Ananas sativus* Schult.), attaquées elles aussi par des *Tylenchidae*.

H. paradoxa n. sp. a été trouvée en un seul point, la station même de Recherches d'Adiopodoumé (près d'Abidjan), en très grande quantité, au voisinage de racines de Mil à Chandelle (*Pennisetum typhoideum* Rich.). Le mâle de *H. paradoxa* n. sp. présente certains caractères aberrants parmi le genre: spicules presque droits, queue conique et non filiforme, gaine spiculaire possédant une languette inférieure. Ces caractères rapprochent beaucoup *H. paradoxa* de *H. longicaudatum* Loos, 1948. THORNE (1955) réexaminant cette dernière espèce trouve que ses caractèristiques sont intermédiaires entre *Hemicycliophora* et *Criconemoides* et qu'elle est donc le type d'un genre non encore décrit. Ici les caractères non typiques du genre concernent uniquement l'appareil sexuel mâle; mais la variation de la forme des spicules chez *Hemicycliophora* est déjà importante entre *H. penetrans* avec ses spicules très longs, presque refermés sur eux-mêmes et *H. typica* où ils ont la forme d'un U largement ouvert,

H. oostenbrinki formant la transition entre ces deux types; on peut fort bien imaginer que *H. paradoxa* représente une variation de nature opposée, vers un spicule droit; il est alors seulement regrettable que le nom de genre ait été choisi en fonction d'une caractéristique qui se révèle si peu constante. D'autre part l'utilisation de la seule structure du mâle pour différencier les genres dans un groupe où l'on ne connaît que 5 espèces bisexuées sur 25 pour *Hemicycliophora* et 2 sur 22 pour *Criconemoides* (cf. RASKI, 1952) semble encore prématurée. Nous aurions en effet rencontré les femelles seules que le moindre doute ne se serait élevé quant à leur appartenance au genre *Hemicycliophora*.

Le parasitisme de ces trois espèces est très probable mais non démontré, car nous n'avons jamais rencontré leurs individus en place sur les racines. Des expériences sont en cours pour démontrer leurs potentialités parasitaires.

Hemicycliophora pauciannulata n. sp.

Femelles: L = 0,740 — 0,896 mm a = 19,8 — 24,2
b = 5,2 — 6,5 c = 11,6 — 13
V = 85,8 — 86,2 %

„Corps marqué de 174-206 anneaux. Cuticule larvaire très lâchement attachée au corps. Champ latéral non marqué sauf dans la partie postvulvaire et simplement par une rupture des stries transverses. Région labiale indiquée par deux anneaux; disque labial représentant $^1/_3$ environ de la largeur labiale. Stylet long de 64,5-68 μ, s'étendant sur 16-17 anneaux; boutons basaux dirigés vers l'arrière. Oesophage typique du genre, s'étendant sur 31-35 anneaux; isthme court, bulbe basal pyriforme. Anneau nerveux très gros, occupant presque toute la longueur de l'isthme. Pore excréteur situé sur le 41ème anneau. Hémizonide net, situé 1 à 2 anneaux en avant du pore excréteur. Utérus avec spermathèque assez développée. Vulve située à 36-46 anneaux de l'extrémité postérieure; repli de la cuticule larvaire formant un tablier très important devant la vulve. Anus situé à 18-22 anneaux de l'extrémité postérieure. Queue conique, régulièrement amincie, extrémité arrondie."

Diagnose: espèce caractérisée par son faible nombre d'anneaux; se différencie de *H. conida* Thorne, 1955 par sa cuticule larvaire nettement détachée du corps, l'absence d'ornementation du champ latéral, la présence d'un hémizonide et d'une spermathèque.

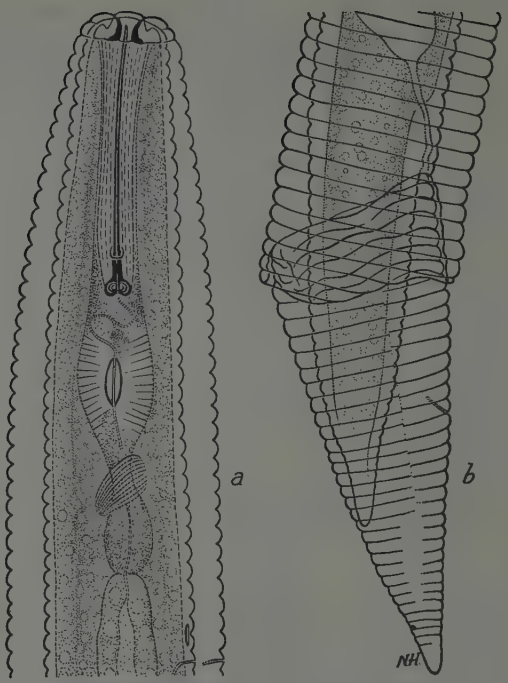

Fig. 1. *Hemicycliophora pauciannulata* n. sp. femelle: a: extrémité antérieure; b: extrémité postérieure (Gross.: × 550).

Localité type: sol de la rhizosphère de *Cocos nucifera* L. atteint de la „maladie de Kaïncopé", Akodessewa, Togo Français.

Holotype: Femelle. Tube N° 65, Laboratoire de Nématologie. I.D.E.R.T. Abidjan, (Côte d'Ivoire).

Hemicycliophora oostenbrinki n. sp.

Femelles: L = 0,744 — 0,888 mm a = 23,1 — 24,9
b = 5 — 5,9 c = 6,7 — 9,4
V = 80,7 — 84,1 %

„Corps marqué de 226-259 anneaux présentant de fines striations longitudinales. Cuticule larvaire très détachée du corps. Champ latéral occupant $^1/_5$-$^1/_6$ de la largeur du corps, marqué par deux rangées parallèles de blocs carrés débutant au niveau du bulbe oesophagien médian et se terminant au niveau de l'anus; antérieurement et postérieurement une seule ligne longitudinale marque le champ latéral.

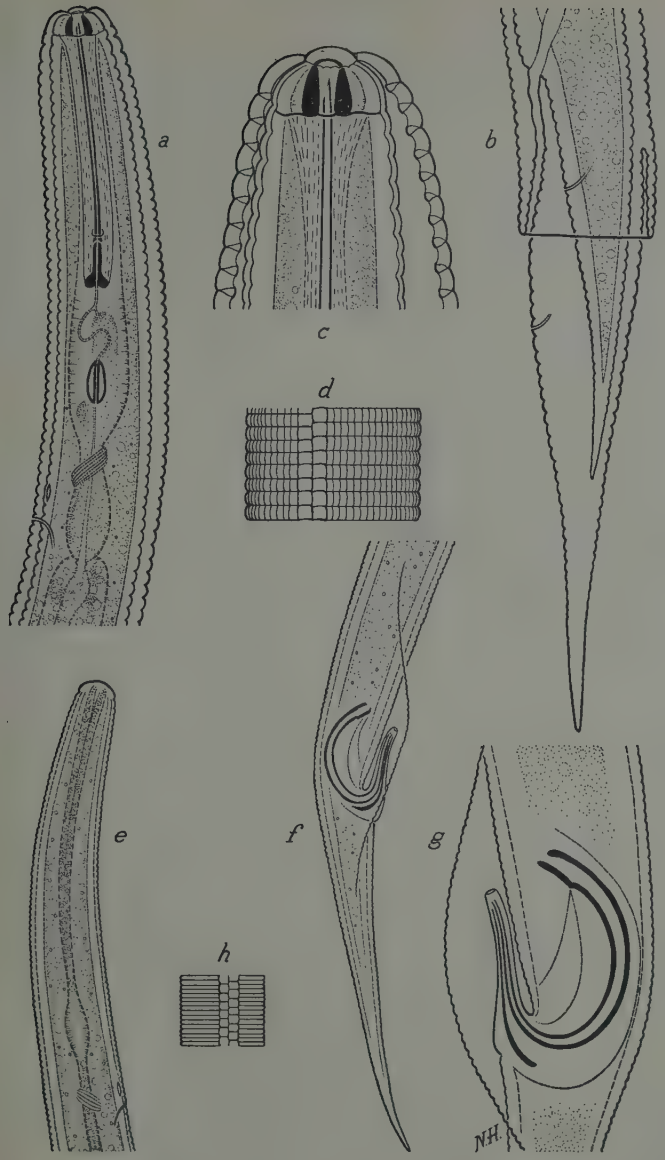

Fig. 2. *Hemicycliophora oostenbrinki* n. sp.; femelle: a: partie antérieure; b: partie postérieure; c: détail de la portion antérieure; d: champ latéral et ornementation des anneaux. Mâle: e: partie antérieure; f: partie postérieure; g: organes sexuels; h: champ latéral. (Gross.: a, b, e, f, g: × 550 — c: × 1130 — d, h: schématique).

Disque labial occupant $^1/_3$ de la largeur labiale. Région labiale arrondie comportant deux anneaux. Stylet de 70-75 μ, s'étendant sur 22-26 anneaux; boutons basaux moyennement développés, dirigés vers l'arrière. Oesophage s'étendant sur 46-51 anneaux, bulbe médian progressivement élargi, isthme court, bulbe basal claviforme. Anneau nerveux assez épais. Pore excréteur situé sur le 43-48ème anneau. Hémizonide situé 3 anneaux avant le pore excréteur et large de 2 anneaux. Ovaire droit sur les exemplaires jeunes, mais pouvant se replier deux fois chez les femelles ayant des œufs développés. Spermathèque présente. Corps nettement rétréci au niveau de la vulve; repli cuticulaire important. Anus difficilement perceptible. Queue conique, effilée. Œufs: 61-66 × 19-26 μ."

Mâles: L = 0,608 — 0,628 mm a = 26,4 — 27,3
b = ? c = 5,9 — 7

„Corps allongé, aminci vers l'avant, longuement effilé vers l'arrière. Cuticule finement annelée (anneaux = 1,2 μ); champ latéral marqué, comme chez la femelle, de deux rangées parallèles de blocs carrés, mais ici un bloc correspondant à plusieurs anneaux; pas de stries sur les anneaux. Tête arrondie, sans expansion de la région labiale. Stylet absent; aucune sclérotisation céphalique visible. Oesophage étroit, bulbe médian très allongé, peu en relief; junction oesophago-intestinale non observée. Pore excréteur à 102-113 μ de l'avant. Anneau nerveux un peu en avant du pore excréteur. Hémizonide distinct, au niveau de l'anneau nerveux. Testicule unique droit. Spicules recourbés, en U très refermé, portion proximale épaisse, portion distale fine; gubernaculum long et fin; gaine spiculaire très développée. Bourse crénelée, fine, se terminant au premier quart de la queue; longueur: environ 3 fois la largeur du corps. Queue longuement conique, extrémité pointue."

Diagnose: l'espèce décrite est proche de *H. membranifer* Micoletzky, 1925 mais en diffère par les caractères suivants: femelles avec un champ latéral plus étroit ($^1/_5$ à $^1/_6$ de la largeur du corps au lieu de $^1/_3$), de ce fait il est constitué de blocs carrés et non rectangulaires; queue plus allongée et régulièrement conique, effilée à l'extrémité. Mâle avec un bulbe oesophagien peu développé mais net et surtout des spicules très refermés, avec un gubernaculum et une gaine spiculaire très développés.

Localité type: Sol autour de racines de Bananier (*Musa paradisiaca* L.); plantation Bardet. Adiopodoumé, près Abidjan, Côte d'Ivoire.

Espèce retrouvée au voisinage des racines de Bananiers et plantains (*M. paradisiaca* L. var. *sapientum*) dans la plupart des plantations de Côte d'Ivoire, ainsi qu'à Kindia [1]) (Guinée Française); également au voisinage de racines d'Ananas (*Ananas sativus* Schult.), à Abobo-Ṭé, près Anyama (Côte d'Ivoire).

Holotype: Femelle: tube N° 64. laboratoire de Nématologie. I.D.E.R.T. Abidjan.

Allotype: Mâle: tube N° 52. Laboratoire de Nématologie I.D.E.R.T. Abidjan.

Hemicycliophora paradoxa n. sp.

Femelles: L = 0,680 — 0,820 mm a = 23,7 — 29,0
b = 4,9 — 5,7 c = ?
V = 78,1 — 84,0 %

„Corps fin, marqué de 256-263 anneaux; cuticule larvaire étroitement accolée au corps. Champ latéral marqué par une simple ligne longitudinale débutant à 4-5 anneaux de l'avant ne persistant pas toujours vers le milieu du corps, mais généralement visible de nouveau dans la région postvulvaire; pas d'autres lignes longitudinales sur les anneaux. Région labiale obtuse; disque labial occupant les $^2/_3$ de la largeur labiale. Stylet long de 61-65 μ et s'étendant sur 23-25 anneaux; boutons basaux plans postérieurement ou très peu dirigés vers l'arrière. Oesophage s'étendant sur 42-45 anneaux; bulbe basal deux fois plus large que l'isthme. Pore excréteur situé au niveau de la junction oesophago-intestinale. Hémizonide situé 3 anneaux en avant du pore excréteur, large de deux anneaux. Utérus avec une très vaste spermathèque allongée. Vulve situé à 50-56 anneaux de l'extrémité postérieure; pas de repli cuticulaire devant la vulve. Ovaire unique généralement droit, pouvant se replier deux fois à l'extrémité proximale; longueur du tractus génital: 361-449 μ (51,5-62,7 % de la longueur totale). Anus vu sur un seul exemplaire: 10 anneaux après la vulve. Queue allongée, d'abord conique puis amincie; extrémité pointue."

Mâles: L = 0,548 — 0,663 mm a = 26,4 — 30
b = ? c = 12,7 — 18,5
T = 28,5 %

„Corps cylindrique, un peu rétréci vers la partie avant, marqué de fines stries (1,3 μ) débutant à 20-22 μ de l'extrémité antérieure. Champ latéral comportant deux lignes simples, continues, parfois indentées à la partie postérieure du corps et occupant $^1/_7$ de la largeur correspon-

1) échantillon envoyé par M. Vilardebo.

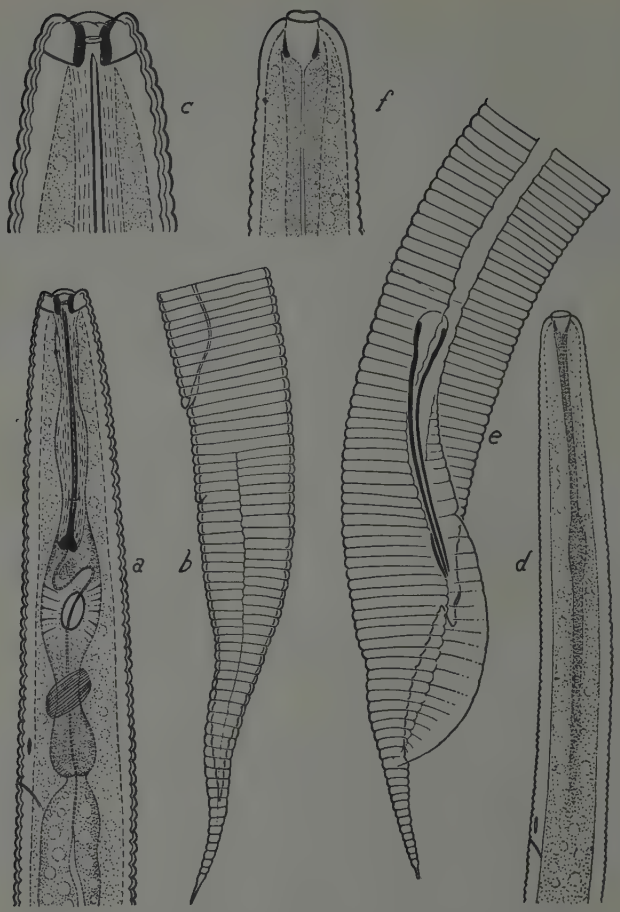

Fig. 3. *Hemicycliophora paradoxa* n. sp.; femelle: a: partie antérieure; b: partie postérieure; c: détail de la portion antérieure. Mâle: d: partie antérieure; e: partie postérieure; f: détail de la portion antérieure. (Gross.: a, b, d,: × 550. c, e, f, : × 1130).

dante. Tête arrondie; lèvres légèrement en relief, soudées. Pharynx avec des parois sclérotisées. Pas de stylet. Oesophage en tube étroit, limites peu nettes. Anneau nerveux non observé. Pore excréteur à 19,5-21,2 % de la longueur totale. Hémizonide large (5 μ), en avant du pore excréteur. Testicule unique droit; spermatozoïdes arrondis, de 2 μ de diamètre. Spicules légèrement courbés à la partie proximale épaissie; partie distale fine et droite; longueur: 30-35 μ. Gubernaculum petit, droit, simple; gaine spiculaire peu développée, languette

inférieure très en relief. Bourse large, crénelée de façon discrète et irrégulière; partie postérieure de la bourse s'étendant sur les $^2/_3$ de la longueur de la queue. Queue conique-filiforme, terminée par une pointe portant parfois une petite boule à l'extrémité."

Diagnose: Les femelles de l'espèce décrite se différencient de celles de *H. micoletzkyi* (Goffart, 1948) Goffart, 1952 par le nombre plus petit d'anneaux (256-263 contre 400) et le stylet plus court (61-65 μ contre 132 μ). Les mâles se différencient de tous ceux connus dans le genre par leurs spicules presque droits.

Localité type: Sol, voisinage de racines de *Pennisetum typhoideum* Rich. — I.D.E.R.T. Adiopodoumé, près Abidjan. Côte d'Ivoire.

Holotype: Femelle. Tube A 121. Laboratoire de Nématologie. I.D.E.R.T. Abidjan.

Allotype: Mâle. Tube N° 95. Laboratoire de Nématologie. I.D.E.R.T. Abidjan.

SUMMARY

Descriptions and figures of three new species of *Hemicycliophora* are given: *H. pauciannulata* of which only females provided with a spermatheca are known, *H. oostenbrinki* and *H. paradoxa,* both with males and females. The three species exhibit a hemizonid; this fact agrees with Thorne's hypothesis on the association of this structure with the sexual process. The males of *H. paradoxa* have nearly straight spicules but the other characteristics are those of the genus.

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(*Accepté: le 1 Juillet 1957*)

Nematologica III (1958): 24-29. E. J. Brill, Leiden.

OBSERVATIONS ON THE EFFECTS OF *DITYLENCHUS DIPSACI* AND *ANGUINA TRITICI* ON CERTAIN WHEAT AND BARLEY VARIETIES

BY

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In 1954 BELLONI reported wheat (*Triticum aestivum* L.) in Italy to be attacked by *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936. He found two varieties, Freccia and Mara were susceptible and three others, Funo, S. 1 and R. 37 resistant. Plants were stunted and yellow, with leaves rolled, curled and twisted in a bizarre manner, stems thickened, internodes short and root system small and easily detached. These symptoms were very similar to those described on wheat by SAVARY (1952) from Switzerland.

In Britain it is generally considered quite safe to grow wheat or barley (*Hordeum vulgare* L.) on land known to be infested with *D. dipsaci* which confines its attacks to oats (*Avena sativa* L.) and rye (*Secale cereale* L.) among the cereals (ANON., 1954). However, as early as 1888 RITZEMA BOS reported wheat as a host of stem eelworm. He found that when sown in soil infested with stem eelworm from rye, a few plants showed symptoms of attack similar to those shown by oats attacked by stem eelworm. Barley was reported as a host of *D. dipsaci* by DARBOUX & HOUARD (1901) and as a host of *Anguina tritici* (Steinbuch, 1799) Filipjev, 1936 by ROFFREDI (1775).

The early symptoms of attack by *A. tritici* on wheat are superficially similar to those described by both BELLONI and SAVARY as due to *D. dipsaci*. This, coupled with the knowledge of the records of both wheat and barley as hosts of *D. dipsaci*, prompted the authors to investigate the behaviour of various wheats (including some Italian varieties), barleys and rye when exposed to infestations of *A. tritici* and of *D. dipsaci* derived from oats.

Methods and Materials

The experiments were set up in 7 inch pots containing John Innes potting compost. The pots were sown with twenty seeds (ten only in

experiment 3), the inoculum added and the seed covered with soil. *D. dipsaci* was extracted from infested oat stubble on a Seinhorst Mistifier (Goodey, 1957), *A. tritici* was added either as galls or as larvae extracted by soaking galls in water. The pots were placed at random in an outside plunge and watered when necessary.

Seed of barley, rye and wheat, including the Italian variety San Pastore, was obtained from the Director, National Institute of Agricultural Botany, Cambridge and of the other Italian varieties used in experiment 3 from the Director, Instituto do Allevemente Vegetale per la Cerealicoltura, Bologna; to both of whom thanks are due. All the varieties of wheat used in these experiments were bread wheats, *Triticum aestivum* L.

Experiment 1. Effects of D. dipsaci *from oats on wheat and barley varieties.*

Four wheat varieties, Atle, Atson, Bersée, and Fylgia, and four barleys, Earl, Freja, Herta and Proctor were tested for their reaction to *D. dipsaci* from oats originating from two different sources in Britain, Perth and Newcastle-on-Tyne.

The experiment was set up on 14/4/55, with duplicate inoculation rates of 0, 5,000 and 50,000 *D. dipsaci* per pot, making a total of forty-eight pots. When five weeks old (19/5/55) the plants were 10-15 cms. high. In each of the pots which had received the higher dose of *D. dipsaci* and also in some of the lower dose series, a few plants showed white patches or lesions on the lower leaves near the midrib and just above the ligule. The symptoms were very similar to those figured by Newton, Hastings & Bosher (1953) for an attack on Berks barley seedlings by *D. dipsaci*. None of the control plants had symptoms.

A plant showing lesions was taken from each pot receiving the higher dose, stained in cotton blue lactophenol, cleared in phenol (Franklin, 1949) and examined microscopically. Adults and pre-adult larvae were present in and around the lesions of all varieties of both wheat and barley. In the wheats Atle and Fylgia and the barleys Herta and Proctor, eggs were also present. The remaining plants were left growing in the pots till 21/7/55, then compared with their respective controls. The basal leaves, which had originally shown lesions, had withered by this time and no fresh symptoms could be seen. There was no noticeable difference in height or general appearance between plants from the inoculated pots and the controls.

Experiment 2. Effects of A. tritici *on barley, wheat and rye.*

This was set up on 9/5/55 to compare the effects of *A. tritici* on twenty-two varieties of barley: Agio, Bere Scotch, Bonus, Camton, Carlsberg, Drost, Earl, Fero, Freja, Haisa II, Heimdal, Herta, HB 34/2/3/33 Bn 251, HB 34/15/2/38 Bn 250, HH 14/4/4 Bn 202, Kema, Kron, Maja, Plumage Archer, Proctor, Rika and Sprat, three of wheat: Atle, Bersée and San Pastore and one rye: Petkus Spring. A single pot of each was sown and inoculated with ten galls of *A. tritici*. In the early stages of growth little distortion of any plants was seen. The stand of all varieties was good except for the wheat San Pastore which was stunted and yellow.

On 22/7/55 ears were taken from each pot and examined in the laboratory. San Pastore wheat had not produced ears by that time but larvae of *A. tritici* were present around the developing ear initial. Bersée wheat had several ears with green galls containing *A. tritici* undergoing their last moult to adults. Atle wheat and Petkus rye were quite free from galls. None of the barleys had galls except one ear of Sprat, where there was a gall, containing one male, one female and many eggs, at the base of an awn.

Experiment 3. Comparison of the effects of D. dipsaci *and* A. tritici *on wheat and barley.*

This was set up on 10/4/56 and was a direct comparison of the effects of *D. dipsaci* and *A. tritici* on wheats and Sprat barley. By this time the Italian varieties of wheat reported on by Belloni and others had been obtained and the following eleven varieties were tested: Atle, Bersée, Fiorello, Fortunato, Freccia, Funo, Mara, R. 37, San Pastore, S. 1 and S. 6. Inoculation in duplicate was carried out by adding ten *A. tritici* galls, 100,000 *A. tritici* larvae or 100,000 *D. dipsaci* per pot. One control pot for each variety was also planted, thus giving eighty-four pots.

After a month no symptoms could be seen on any of the pots, but after ten weeks (19/6/56) all the wheat pots inoculated with *A. tritici* were showing crinkled leaves and twisted stems. None of the *D. dipsaci* series of pots showed symptoms of eelworm attack nor did they later. On 11/9/56, when any ears produced had ripened, a final examination was made. The growth of Fiorello had been very poor in both inoculated and control pots and a few, poor, blind ears only had been produced. The ears from all the *A. tritici* series of pots were collected,

TABLE I

Effects of A. tritici *and* D. dipsaci *on eleven varieties of wheat and one of barley*

Variety	A Control No. of ears	B 10 *A. tritici* galls/pot No. of ears	% ears galled	I	C 100,000 *A. tritici* larvae/pot No. of ears	% ears galled	I	B & C % ears galled	I	D 100,000 *D. dipsaci*/pot No. of ears
Atle (Wheat)	10	11	27	0.3	11	18	0.2	35	0.3	13
		12	25	0.3	10	50	0.5			10
Bersée (W)	9	7	0	—	12	17	0.2	21	0.3	8
		8	50	0.6	11	18	0.2			13
Fiorello (W)	3	6	17	0.2	5	60	0.2	39	0.6	7
		5	80	1.6	5	—	—			10
Fortunato (W)	9	15	33	0.3	12	33	0.5	26	0.3	12
		14	29	0.3	11	9	0.1			12
Freccia (W)	13	18	78	1.0	11	27	0.3	42	0.6	9
		15	33	0.7	10	30	0.3			14
Funo (W)	13	14	50	0.8	9	67	0.9	55	0.8	9
		9	33	0.3	10	70	1.1			12
Mara (W)	10	10	70	0.7	13	69	0.7	69	0.8	12
		17	82	1.3	13	54	0.5			13
R. 37 (W)	9	9	56	0.6	12	33	0.3	41	0.4	10
		9	44	0.6	7	29	0.3			13
San Pastore (W)	10	4	100	2.0	3	100	1.0	90	1.2	10
		9	78	0.8	11	82	0.8			9
S. 1 (W)	9	8	100	2.0	14	86	1.7	89	1.9	11
		10	100	2.0	10	70	0.9			12
S. 6 (W)	10	8	62	1.3	10	50	0.9	54	0.9	11
		9	89	1.4	7	14	0.6			12
Sprat * barley	16	26	0	0	17	0	0	0	0	17
		20	0	0	24	0	0			16

* 20 seeds/pot.

the percentage galling recorded, and each ear was assessed for galling in arbitrary units as follows:

Rating	Category
0	No galls
1	Few galls
2	Many galls (ears "scary")

An index of infestivity (I) was then calculated for each pot according to the formula:

$$I = \frac{n_0R_0 + n_1R_1 + n_2R_2}{n}$$

where n = total number of ears per pot,
n_0, etc = number of ears in each category,
R_0, etc = Rating of each category.

The results of this examination are presented in Table I.

DISCUSSION

In experiment 3 the high dose of 100,000 *D. dipsaci* per 7 inch pot (which causes very severe damage to oats) failed to cause any noticeable damage to wheat, such as that described by BELLONI for some of the same varieties. This suggests that the race of stem eelworm attacking wheat in Italy is biologically different from the oat race of Britain. From correspondence with Dr. SAVARY it appears that in Switzerland oats, rye, wheat and barley are all attacked by *D. dipsaci* and that *A. tritici* is rare. This adds further evidence of races of *D. dipsaci* capable of attacking wheat outside Britain. However, the results of experiments 1 and 3 indicate that in Britain there is no real danger from growing wheat or barley on a soil highly infested with the oat race of stem eelworm. Experiment 2 confirms the fact that barley is, for all practical purposes, not attacked by *A. tritici*.

BELLONI found that the Italian wheat varieties Funo, R. 37 and S. 1 were resistant to attack by *D. dipsaci*. In the experiments here described these and all the other wheats tested were susceptible to *A. tritici*. Thus resistance to *D. dipsaci* does not infer resistance to *A. tritici*.

ZUSAMMENFASSUNG

Auf Grund eines Berichts von BELLONI (1954), dass in Italien Weizen (*Triticum aestivum* L.) von *Ditylenchus dipsaci* angegriffen wird, wurden Weizen-, Gerste- und Hafersorten auf ihre Anfälligkeit gegenüber *Ditylenchus dipsaci* von Hafer aus Gross-Britannien und auf *Anguina tritici* geprüft. Keine Sorte wurde von *D.*

dipsaci befallen, aber alle Weizensorten, einschliesslich einiger italienischer, waren gegenüber *A. tritici* anfällig. Es scheint, dass die *D. dipsaci*-Rasse, die in Italien Weizen angreift, von der Haferrasse Gross-Britanniens biologisch verschieden ist. In Gross-Britannien besteht keine Gefahr eines Angriffs von *D. dipsaci* an Weizen oder Gerste. Resistenz des Weizens gegenüber *D. dipsaci* bedeutet aber keine Resistenz gegenüber *A. tritici*.

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(*Received for publication: July 10, 1957*)

Nematologica III (1958): 30-33. E. J. Brill, Leiden.

AN INOCULATION TRIAL WITH *PRATYLENCHUS PENETRANS* IN POTATOES

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Symptoms of sickness in potatoes due to the root infesting nematode *Pratylenchus penetrans* (Cobb, 1917) Sher et Allen, 1953 were recorded by the author in 1954. A positive correlation between damage and eelworm density in the roots was found and other circumstantial evidence indicated that the eelworm was the causal agent. This has been confirmed by further crop rotation and soil disinfection studies (unpublished) and also by the following inoculation experiment. Earlier inoculations had already shown that *P. penetrans* was the primary cause of sickness symptoms in apple and other woody perennials (Oostenbrink 1955, Parker & Mai 1956).

METHODS AND RESULTS

The methods used for the collection of nematodes from roots and soil are those developed by the author (c.f. s'Jacob & Stemerding 1956). A large number of *P. penetrans* were extracted from potato roots obtained from an infested field. The eelworm suspension was washed several times with tap water and finally consisted of about 83 % *P. penetrans*, 2 % other *Tylenchida* and 15 % saprozoic nematodes.

Four 8 litre-pots were filled with sandy soil which had been steam-sterilized several months before. Two pots were inoculated at the rate of 9000 *P. penetrans* each, in two others a quantity of the suspension water was poured from which the nematodes had been removed by repeated sieving over a 50 μ sieve. The inoculation took place on the 22nd of July 1954 and four tuber pieces of the potato, variety Voran, were planted in each pot the next day. The plants developed well and did not show any effect from the inoculation. They were lifted in December and at that time the nematode population of each pot was determined both within the plant roots and externally in a small soil sample. The extracted nematodes were re-inoculated into the pot soil.

In 1955 the same potato variety was again planted in the pots and a marked growth retardation was noticed in the inoculated plants (Fig. 1). The plants were lifted and evaluated in September and the nematode population was determined as in the preceding year.

Fig. 1. Potatoes, variety Voran in July 1955. Steam-sterilized soil; two pots on the right were inoculated with *Pratylenchus penetrans* (c.f. Table 1)

Table I summarizes data on plant growth and nematode populations, calculated per pot. Identification of 20 specimens under high magnification confirmed, that the *Pratylenchus* in Col. 4 were identical with the inoculated species, viz *P. penetrans*. The secondary nematodes include other tylenchids and the saprozoic forms.

DISCUSSION AND CONCLUSION

Inoculation of the nematodes caused typical symptoms of growth retardation in the second year. This must be due to the nematodes and notably to *P. penetrans*, since this was the only plant parasitic species present in high numbers and the only one in which the population responded to the crop.

Col. 2 of Table I reveals that the number of *Pratylenchus* after the first potato crop was much less than the inoculated dose. This decrease is considered significant, even when allowance is made for the fact that the extraction was not complete. It is probably because many of the *Pratylenchus* specimens did not recover from the process of extraction, cleaning and inoculation, as has been recorded earlier (1954). After the second crop, the number of *Pratylenchus* had in-

TABLE I

Results of inoculation of 8 liter-pots of steam-sterilized soil with Pratylenchus penetrans. Potatoes in 1954 and 1955; in 1954 no growth inhibition was noticed.

1	2			3				4		
Number of *Pratylenchus* added	Number of *Pratylenchus* per pot; between brackets secondary nematodes; December 1954			Weight of potato plants in g; September 1955				Number of *Pratylenchus* per pot; between brackets secondary nematodes; September 1955		
July 1954	Soil	Roots	Total	Haulms	Roots	Tubers	Total	Soil	Roots	Total
0	*0* (107750)	*7* (290)	*7* (108040)	62	28	395	485	*200* (62500)	*882* (4074)	*1082* (66574)
0	*0* (116083)	*3* (165)	*3* (116248)	72	48	420	540	*0* (53000)	*1416* (5448)	*1416* (58448)
9000	*1000* (73833)	*180* (618)	*1180* (74451)	22	20	165	207	*96000* (55000)	*56000* (30000)	*152000* (85000)
9000	*1333* (86000)	*125* (289)	*1458* (86289)	34	19	233	286	*92500* (44500)	*96900* (33250)	*189400* (77750)

creased to 17 and 21 times the inoculated dose respectively. The data suggest that a much greater rate of increase must have occurred from the time when the infection was established. The uninoculated pots also became slightly infected with *Pratylenchus*, naturally during the trial or accidentally at the time of inoculation.

The foregoing data may help to explain the negative result of inoculations with *P. penetrans* in peas (SEINHORST 1954) and in strawberries (RASKI 1956) whereas both plant species are considered to be susceptible to damage by the author and other workers. Sampling techniques are evidently indispensible in this work and also the influence of an inoculation should be tested over at least two years, as suggested by RASKI (1956).

ZUSAMMENFASSUNG

Die Inokulation von *Pratylenchus penetrans* verursachte im zweiten Zuchtjahr eine typische Wachstumshemmung bei Kartoffeln (Fig. 1). Die Nematodenpopulation war nach dem ersten Kartoffelanbau viel kleiner als die Inokulation, was der Empfindlichkeit der Nematoden infolge der Behandlung zugeschrieben wird. Nach dem zweiten Kartoffelanbau betrug die *Pratylenchus*population mehr als das 17- resp. 21-fache der ursprünglichen Inokulation (siehe Tab. 1).

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(Received for publication: July 25, 1957)

Nematologica III (1958): 34-43. E. J. Brill, Leiden.

DIE IDENTITÄT VON *TYLENCHUS ROBUSTUS* DE MAN

VON

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Als FILIPJEV im Jahre 1936 [1]) seine neue Gattung *Rotylenchus* aufstellte, bezeichnete er *Tylenchus robustus* DE MAN, 1880 als Species typica dieser Gattung. Die Identität dieser Art stand bisher nicht fest (siehe STEINER 1945) und damit war auch die Deutung des Namens *Rotylenchus* unsicher. Durch Untersuchung von Präparaten der Sammlung DE MAN und von Bodenproben der Typelokalität haben wir versucht, dieses Problem zu lösen.

Tylenchus robustus wurde von DE MAN beschrieben im Jahre 1876 und nachher in seinen Arbeiten über die Nematoden der niederländischen Fauna aus 1880 und 1884.

Nach der Beschreibung aus dem Jahre 1876 hat die Art eine deutlich abgesetzte Lippengegend mit eigentümlich gefärbtem innerem Skelett. Die Länge des Tieres wird mit 1,1 mm angegeben, die Vulvalage mit 165/281 = 58,7 % und die Länge des Mundstachels mit 10/255 mm = 39 μ. Der Ösophagus wird abgebildet mit „tylenchoidem" Terminalbulbus (STEINER 1945), aber dies hat keine besondere Bedeutung, weil die Gestalt des hinteren Ösophagusteils sehr stark wechselt je nach der Seite, von der aus man ihn betrachtet, wie THORNE 1949 gezeigt hat. Es wurden nur Weibchen gefunden. Die Art soll in tonigen Wiesenböden in der Umgebung der Stadt Leiden ziemlich häufig sein.

1880 sagt DE MAN, die Lippengegend sei hoch und vom Körper abgesetzt; das Kopfskelett zeige eine gelbliche Färbung. Die Stachellänge sei $^1/_4$-$^1/_5$ der Ösophaguslänge. Für β gibt der Autor beim ♂ 5-6,

1) Als Jahreszahl der Aufstellung der Gattung *Rotylenchus* soll 1936 beibehalten werden. Zwar hatte FILIPJEV schon 1934 den Namen *Rotylenchus* veröffentlicht, aber nach den Internationalen Nomenklaturregeln (Art. 25) hat ein nach dem 31. Dezember 1930 veröffentlichter neuer Gattungsname nur dann nomenklatorische Gültigkeit, wenn er u.a. mit einer Diagnose versehen ist. *Rotylenchus* FILIPJEV 1934 genügt dieser Bedingung nicht und ist demnach *nomen nudum*.

beim ♀ 8; für die Länge des ♂ 1,3 mm, des ♀ 1,7 mm. Für die Stachellänge ergibt sich somit beim ♂ mindestens 43 μ, beim ♀ 42,5-53 μ. (Wie De Man die Ösophaguslänge gemessen hat, ist in diesem Zusammenhang gleichgültig). Die Vulva liege sehr wenig hinter der Körpermitte. Der männliche Schwanz trage eine postanale Papille auf jeder Seite. Bezüglich des Vorkommens wird gesagt: Eine ziemlich häufige Art, welche sowohl die feuchte, von süssem oder brackigem Wasser getränkte Erde unserer Wiesen wie den sandigen Dünenboden bewohnt.

Die Beschreibung aus dem Jahre 1884 ist der aus 1880 nahezu gleich, es werden aber einige Ergänzungen hinzugefügt. Die weiblichen Geschlechtsorgane seien schon bei 0,8 mm langen Exemplaren voll entwickelt. Es werden weiterhin u.a. ein ♀, ein weiblicher Kopf und ein männlicher Schwanz abgebildet. Die Zeichnung des Weibchens aber weicht in mehreren wesentlichen Punkten von der Beschreibung ab. Das Tier ist viel zu klein, die Lippengegend ist kaum abgesetzt, die Vulva liegt bei 60 % und der Stachel misst 32 μ. Dieses Bild stellt unzweifelhaft nicht die beschriebene Art dar. Der männliche Schwanz dagegen stimmt mit der Beschreibung gut überein.

Im Jahre 1932 veröffentlichte Goodey eine ausführliche Beschreibung von *Anguillulina robusta* (De Man). Seine Art zeigt folgende Merkmale: Lippengegend kaum gegen den Körper abgesetzt, Stachellänge 28-32 μ, Vulva bei 52-65 %. Er fand nur ♀♀, übernahm aber Beschreibung des ♂ und Abbildung des männlichen Schwanzes von De Man. Die von Goodey gefundenen ♀♀ weichen also erheblich von den von De Man beschriebenen Exemplaren ab.

1949 gab Thorne eine breitere Diagnose der Gattung *Rotylenchus* und eine Neubeschreibung von *R. robustus*. Die Beschreibung stimmt im wesentlichen mit der von Goodey überein; Thorne erwähnt *Anguillulina robusta* (De Man) Goodey in der Synonymie. Die Gattung hat nach Thorne eine nicht abgesetzte Lippengegend, deren Kutikula nicht in Blöckchen zerteilt ist. Für die Körperlänge von *R. robustus* wird angegeben: ♂ 0,8-1,0 mm; ♀ 1,0-1,2 mm. Die Stachellänge wird nicht angegeben, die zwei abgebildeten Stacheln messen 32,6 und 34,1 μ. Die laterale Papille auf dem männlichen Schwanz liege präanal oder adanal; dies stimmt also nicht, wie Thorne selber bemerkt, zu der Beschreibung und Abbildung von De Man. Nach Thorne haben De Man bei der Beschreibung von *Tylenchus robustus* mehrere Arten vorgelegen (was zweifellos richtig ist, siehe oben), vielleicht auch Stücke der von Thorne in demselben Aufsatz beschrie-

benen neuen Art *Hoplolaimus uniformis*. Die Gattung *Hoplolaimus* Von Daday hat nach Thorne folgende Merkmale: Lippengegend deutlich gegen den Körper abgesetzt, mit gelblichem innerem Skelett und durch Längsstreifen und Querringelung in unregelmässige Blöckchen zerteilter Kutikula; Gubernaculum mit Titillae. Für *H. uniformis* gibt Thorne eine Körperlänge von 1,4 mm an. Über die Länge des Mundstachels werden keine Angaben gemacht; für das abgebildete Exemplar lässt sich ein Wert von 47 μ errechnen. Die Stücke, nach denen die Art beschrieben wurde, stammten aus den Niederlanden (Thorne, briefliche Mitteilung).

1954 gab Oostenbrink eine ergänzende Beschreibung von *Hoplolaimus uniformis*. Die Körperlänge wird von diesem Autor angegeben mit: ♀ 1,17-1,62 mm; ♂ 1,04-1,26 mm. Der Stachel misst nach ihm rund 50 μ beim ♀, rund 45 μ beim ♂. Oostenbrink zeichnet wie Thorne die laterale Schwanzpapille des ♂ postanal.

Der oben erwähnten Auffassung von *Rotylenchus robustus* als einer Art mit nicht abgesetzter Lippengegend und einer Stachellänge von 28-34 μ hat auch Golden (1956) sich in seiner Gattungsrevision angeschlossen.

Nach dem oben Gesagten ist es jedoch klar, dass die Richtigkeit dieser heutzutage allgemein akzeptierten Deutung zweifelhaft ist, was u.a. schon Steiner (1945) vermutete.

MATERIAL

Durch die Freundlichkeit des Herrn Prof. Dr. H. Engel vom Zoologischen Museum der Universität Amsterdam steht uns De Man's alte Sammlung zur Verfügung. Diese Sammlung ist aus zwei Teilen zusammengesetzt, die sich voneinander unterscheiden durch Grösse der Objektträger und Deckgläser, durch die verschiedenen Typen der Schachteln, in denen De Man die Präparate aufbewahrte, sowie durch den Umstand, dass die Präparate der einen Abteilung schwarz, die der anderen gelb umrandet sind. Die erstgenannte Abteilung ist von De Man selber katalogisiert worden. Wie mit grosser Wahrscheinlichkeit aus seinen Notizbüchern, sowie aus den vorhandenen Fundort- und Datumangaben hervorgeht, handelt es sich hier um das Material, auf das De Man seine Veröffentlichungen aus 1876, 1880, 1881 und 1884 über die Nematoden der niederländischen Fauna basiert hat. Bis auf einige Arten, die gar nicht veröffentlicht sind, werden alle darin enthaltenen Arten in den genannten vier Abhandlungen beschrieben, während die später beschriebenen Arten sowie die in späteren Jahren (auch in den Niederlanden) gesammelten Exemplare bekannter Arten

in der zweiten Abteilung untergebracht sind. Bei einigen Arten, die hierauf nachgeprüft wurden, stimmten die in den Präparaten enthaltenen Stücke gut mit den Beschreibungen in den erwähnten Veröffentlichungen überein. Nur wenige Präparate dieser Sammlung sind mit Datum oder Fundort versehen. Die 33 datierten sind sämtlich aus dem Jahre 1879.

In dieser Abteilung fanden sich 4 Präparate mit der Bezeichnung *Tylenchus robustus* (in De Man's Handschrift). Jedes Präparat enthielt ein Exemplar. Die Älchen waren mit einer Kruste überdeckt, die genaue Beobachtungen der Einzelheiten unmöglich machte. Diese Kruste liess sich aber leicht lösen in Alkohol 30 %, wonach die Tiere in der gebräuchlichen Weise über F. A. A. und Alkohol-Glyzerin eingeschlossen werden konnten. Sie befinden sich noch in ziemlich gutem Zustande.

Präparat Nr. 208 enthielt ein Weibchen mit folgenden Massen: L 1622 μ; V 51,9 %; α 31,7; β (nach De Man gemessen, d.h. bis zum Hinterrand des Ösophagus-Terminallappens) 7,9; γ 58,9; Stachel 46,0 μ. Die Lippengegend war abgesetzt, der Schwanz kurz und breit abgerundet. Phasmide nicht mehr deutlich zu unterscheiden, aber ziemlich gross, da die inneren Seitenlinien dort auseinanderbogen. Kutikula der Lippengegend in unregelmässige Blöckchen zerteilt, genau wie es Thorne (1949) in seiner Fig. 1 B für *Hoplolaimus uniformis* abbildet.

Präparat Nr. 209 enthielt ein Männchen mit folgenden Massen: L 1253 μ; α 31,8; γ 42,3; Stachel 42 μ. Lippengegend abgesetzt, hoch und abgerundet, Kutikula in Blöckchen zerteilt. Die Einzelheiten des Schwanzes wegen der schrägen Lage nicht sehr deutlich. Es konnte aber doch festgestellt werden, dass die Phasmide postanal gelegen war und dass das Gubernaculum mit Titillae versehen war.

Aus De Man's Notizen geht hervor, dass diese beiden Stücke aus Dünenboden bei Katwijk, März 1879 gesammelt wurden. De Man bemerkt selber, der Kopf des Männchens sei hoch und halbkugelförmig.

Zu den erwähnten Massen muss bemerkt werden, dass der Wert für α zu niedrig ist. Da De Man seine Deckgläser nicht durch gläserne Stäbchen zu stützen pflegte, sind alle Nematoden in seiner Sammlung mehr oder weniger plattgedrückt.

Die beiden übrigen Präparate enthielten Larven, die nicht mit Sicherkeit zu bestimmen waren. Nr. 57 war eine Larve mit abgesetzter Lippengegend, kurzem und breit gerundetem Schwanz und deutlicher Phasmide; Stachellänge 30,8 μ. Die Feinstruktur der Lippengegend war nicht mehr zu sehen. Wahrscheinlich aber gehört dieses Exemplar zur

selben Art wie die vorhergehenden Stücke. Nr. 58 war eine Larve mit asymmetrisch abgerundetem Schwanz und nicht abgesetzter Lippengegend; Stachellänge ebenfalls 30,8 μ. Sie gehört einer anderen, nicht näher zu determinierenden Art an.

De Man's Stücke von *Tylenchus robustus* stammten aus der Umgebung von Leiden, und zwar aus tonigen Wiesenböden (1876, 1880, 1884) und aus sandigen Dünenböden (1880, 1884). Die in den letzten fünf Jahren angestellten Untersuchungen von Hunderten von Bodenproben haben ergeben, dass *Hoplolaimus uniformis* in den sandigen und mittelschweren Böden der Äcker und Gärtnereien in der Umgebung von Leiden sehr allgemein vorkommt. In den tonigen Wiesen aber kommt er dort kaum oder gar nicht vor. Um dies nochmals zu prüfen, untersuchten wir 22 speziell zu diesem Zwecke genommene Wiesenproben. *Hoplolaimus uniformis* wurde in nur einer Probe aufgefunden, die nicht einer alten Wiese entnommen war. In den anderen Proben wurde keine Art aus dieser Gruppe mit abgesetzter Lippengegend gefunden, wohl aber allgemein *Tylenchorhynchus maximus* Allen, *Helicotylenchus erythrinae* (Zimmermann) und eine mit *Anguillulina robusta* (De Man) Goodey verwandte *Rotylenchus*-Art.

DISKUSSION UND SCHLUSSFOLGERUNGEN

Die zwei erwachsenen Exemplare in den Präparaten Nr. 208 und 209 gehören zweifellos zu Thorne's Art *Hoplolaimus uniformis*. Weiterhin entsprechen sie De Man's Beschreibung aus 1880 (wichtig sind in dieser Hinsicht Stachellänge, Gestalt und Farbe der Lippengegend, und die Lage der Phasmide beim ♂). Da die 1880 von De Man beschriebene Art Genotypus von *Rotylenchus* ist, soll sie auf jeden Fall diesen Gattungsnamen tragen.

Filipjev hat im Jahre 1936 die Gattung *Rotylenchus* zweimal veröffentlicht. 1936-A sagt er: Typus *Tylenchus robustus* De Man 1880, ohne weiteres; einige Zeilen weiter bemerkt er aber, dass die Gattung *Hoplolaimus* sich von *Rotylenchus* unterscheidet durch das Fehlen der lateralen Papillen auf dem Schwanz des ♂. Die ♂♂ der Goodey'schen Art haben aber präanale Phasmiden, *uniformis* dagegen postanale. Es wird also hier auf *uniformis* hingewiesen (Vgl. Steiner 1945).

1936-B gibt auch keine spezifischen Merkmale an, nur gibt der Autor eine Abbildung eines ♀ Vorderendes und eines ♂ Schwanzes, beide aus Goodey 1932 übernommen. Wie oben gesagt, beziehen diese sich aber auf zwei verschiedene Arten.

1936-A trägt das Datum 22. Juli. 1936-B ist (nach brieflicher Mit-

teilung von Frau Dr. Sveshnikova) ebenfalls im Monat Juli erschienen; das genaue Datum war leider nicht festzustellen. Jedenfalls ist klar, dass De Man's Beschreibung aus 1880 als massgebend betrachtet werden muss.

Es erhebt sich nun die Frage, ob De Man 1876 dasselbe Tier beschrieben hat wie 1880 oder nicht. Im ersten Falle wäre der Name *uniformis* Thorne aus Prioritätsgründen durch *robustus* De Man zu ersetzen, im letzten aber wären *robustus* De Man und *uniformis* Thorne zwei verschiedene Arten; letztere wäre Genotypus von *Rotylenchus*.

Die Beschreibung aus 1876 ist nicht sehr vollständig und enthält einige rätselhafte Angaben, die jedoch verständlich werden, wenn wir annehmen, De Man habe schon damals mehrere Arten, unter welchen *uniformis*, synonymisiert und als *T. robustus* bezeichnet [1]).

So sagt er, die Art sei in Wiesen ziemlich häufig, wiewohl *H. uniformis* entschieden kein Wiesenbewohner ist.

Zweitens gibt er die Länge des *robustus* mit 1,1 mm an. Aus dem Texte seiner Veröffentlichungen geht hervor, dass er nur die maximalen Werte der Körperlänge anzugeben pflegte, wie schon Micoletzky 1917 bemerkt hat. Nun ist eine Körperlänge von 1,1 mm für *uniformis* nur wenig. Hätte De Man nur diese Art (ziemlich häufig!) beschrieben, so hätte er gewiss grössere Stücke finden müssen. Er hat also sicher nur wenige Exemplare von *uniformis* gesehen, vielleicht sogar nur eins.

Drittens gibt er an, er habe Exemplare mit 3-4 Eiern im Uterus gesehen. Diese Zahl ist für *uniformis* nicht normal, und 1880 sagt De Man selbst: im Uterus wurde ein ziemlich grosses Ei beobachtet. Vielleicht waren es Weibchen einer anderen Art, die 3-4 Eier trugen.

Viertens hatte De Man damals nur Weibchen gefunden, obwohl die Männchen von *uniformis* gar nicht selten sind.

Dass De Man doch auch *uniformis* gesehen hat, geht aus seinen Angaben über Gestalt und Farbe der Lippengegend und über die Länge des Mundstachels hervor: *uniformis* ist die einzige bekannte Art, deren Stachel eine Länge von 40 μ erreicht. Zwar ist 30 μ eigentlich zu wenig (der kürzeste von uns beobachtete Stachel mass 41 μ), aber die Ab-

1) Wie schon bemerkt, haben De Man auch später bei seinen Beschreibungen mehrere Arten vorgelegen. Die Abb. 62 aus 1884 könnte die oben erwähnte, mit *A. robusta* verwandte Art darstellen; dies würde auch seine Behauptung erklären, bei *Tylenchus robustus* seien die weiblichen Gonaden schon bei 0,8 mm langen Tieren voll entwickelt, was für *uniformis* keineswegs zutrifft.

bildung legt die Vermutung nahe, dass De Man die feine Spitze des Stachels nicht gesehen hat und demnach der Stachel länger ist als von ihm angegeben. Die Abbildung stellt ohne Zweifel die Thorne'sche Art dar.

Diese Auffassung, dass De Man 1876 *uniformis* nebst anderen Arten vorgelegen haben, hat ohnehin mehr für sich als die andere Möglichkeit, dass *Tylenchus robustus* De Man 1876 eine von *Hoplolaimus uniformis* Thorne verschiedene Art ist. Sie soll ja in der Nähe von Leiden häufig vorkommen und dennoch wurde ausser *uniformis* keine Art aufgefunden, die der Beschreibung von 1876 entspricht. Dass im übrigen De Man's Beschreibungen von 1876, 1880 und 1884 sich auf *uniformis* basieren, obgleich ihm Artgemische vorlagen, erklärt sich aus der schon erwähnten Tatsache, dass er immer die grössten Stücke für seine Artbeschreibungen auszuwählen pflegte.

Wir sind also der Ansicht, *Tylenchus robustus* De Man 1880 müsse als Synonym zu *Tylenchus robustus* De Man 1876 betrachtet werden. Der Thorne'sche Artname *uniformis* kommt infolgedessen als Synonym zu *robustus* in Wegfall.

Das Weibchen in Präparat Nr. 208 kann, da erst 1879 gesammelt, nicht die Holotype sein. Wir dürfen es aber als Metatype bezeichnen.

1880/1884 gibt De Man als Masse des ♂: L 1,3 mm; α 35; β 5-6; γ 40. Diese Werte stimmen gut überein mit denen des ♂ aus Präparat Nr. 209, was es um so wahrscheinlicher macht, dass dies eins der Stücke ist, nachdem die (erste!) Beschreibung erfolgt ist. Wir bezeichnen es deshalb als Allolectotype.

Anguillulina robusta (De Man) Goodey, 1932, und 1940, syn. *Rotylenchus robustus* (De Man) and Thorne 1949, Goodey 1951 und Golden 1956, muss nun einen neuen Artnamen erhalten, da erwiesen ist, dass dies nicht die von De Man beschriebene Art ist. In der Literatur finden sich folgende Synonyme zu *robustus*:

1. *Tylenchus dihystera* Cobb, 1893 apud Goodey, 1932 und 1940 und Filipjev & Schuurmans Stekhoven, 1941. Masse: L 850 μ, α 27, γ 40, Stachel 28-30 μ. Leider gibt Cobb keine Abbildung. Bezüglich des Schwanzes sagt er aber: "The ventral contour of the conoid tail was continuous with that of the belly, there being no bend or curve as on the dorsal side". Dies spricht wider die Identität mit *robustus* auctt. Weiterhin sagt Cobb: "the anus was inconspicuous", während Goodey 1932 im Gegenteil bemerkt: "rectum and anus distinct". Die Art ist daher wohl von *robusta* Goodey verschieden, wiewohl Goodey 1940 sie für identisch erklärt. Wir bevorzugen aber, *Tylenchus dihystera* Cobb als Species inquirenda zu betrachten.

2. *Tylenchus olaae* Cobb 1906 apud Micoletzky 1922 und Filipjev & Schuurmans Stekhoven 1941. Masse: L 720 μ, V 63 %, α 25,6; γ 200. Auch diese Art ist nach der Beschreibung nicht genau zu deuten. Cobb sagt auch hier: "the anus is inconspicuous". Über die Gestalt des Schwanzes (die übrigens von den Autoren sehr verschieden interpretiert wird) bemerkt er: "The tail is convex conoid to the rather blunt terminus". Auch *olaae* Cobb ist somit bestimmt nicht synonym zu *robusta* Goodey: diese Art nat ja einen breit gerundeten Schwanz ohne eigentliche Spitze.

3. *Aphelenchus steueri* Stefanski 1916, apud Micoletzky 1922. Masse: L 1103 μ; α 41; β (bis zum Hinterrande des Mittelbulbus) 11,75; γ 58. Die Abbildung zeigt ein stark verschmälertes Vorderende. Die Länge des abgebildeten Stachels ist 15 mm, des Ösophagus (in obengenannter Deutung) 42 mm; die wirkliche Stachellänge ist somit 1103 μ: 42/15 × 11,75 = 33,5 μ. Das Tier ist aber viel schlanker als die Goodey'sche Art. Der Schwanz ist unregelmässig buckelig gezeichnet. Die Art wurde aus Süsswasser (Inn-Fluss) beschrieben. Es ist nicht ganz ausgeschlossen, dass sie doch mit *robustus* auctt. identisch wäre. Dies lässt sich jedoch heutzutage nicht mit Sicherheit entscheiden; *Aphelenchus steueri* ist am besten einstweilen als Species inquirenda zu führen.

4. *Tylenchorhynchus robustus* var. *brevicaudatus* Micoletzky 1922 apud Micoletzky 1922, Goodey 1932 und 1940, Filipjev & Schuurmans Stekhoven 1941. Unter den Namen *Tylenchorhynchus robustus* fasst Micoletzky 1922 eine grosse Anzahl Formen zusammen. Die von ihm neu aufgestellte var. *brevicaudatus* hat eine leicht abgesetzte Lippengegend und ist in Wiesenböden sehr gemein. Der in Fig. 55-i abgebildete Stachel misst aber etwa 58 μ [1]) und die Verhältnisse des Vorderendes sind deutlich verschieden von denen der Goodey'schen Art (sie nähern sich denen von *T. robustus* De Man); die S. 610 gegebenen Längenangaben dagegen stimmen damit gut überein. Die Stachellänge ist aus den Massen nicht genau zu errechnen. Die Var. dürfte ebenfalls mehrere Formen umfassen. Jedenfalls ist der Name *brevicaudatus* nicht ohne weiteres als Synonym zu *robustus* auctt. zu betrachten.

Da also T. Goodey anscheinend der Erste war, der nachweislich diese Art beschrieben hat, schlagen wir vor, sie nach ihm *goodeyi* zu benennen. Die in der Literatur vorhandenen Längenangaben dieser Art sind kritisch zu betrachten, weil einige Autoren für die obere Grenze

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1) Siehe Micoletzky's Fussnote auf Seite 630!

den Wert 1,7 mm von De Man abgeschrieben haben. De Man's Art ist aber, wie gesagt, eine andere.

Es ist sehr wahrscheinlich, dass viele Literaturangaben von *Rotylenchus robustus* sich entweder auf *goodeyi* oder auf noch andere Arten beziehen.

Die Berichtigung der Deutung von *Tylenchus robustus* De Man hat noch weitere nomenklatorische Änderungen zur Folge. Mit diesen wird sich der nachstehende Artikel von Andrássy befassen.

Es ergibt sich somit:

Gattung: *Rotylenchus* Filipjev 1936

Typus generis: *Rotylenchus robustus* (De Man 1876 apud De Man 1880) Filipjev 1936

Synonyme: *Tylenchus robustus* De Man 1876
Tylenchus robustus apud De Man 1880, 1884, 1917
Anguillulina robusta ♂ apud Goodey 1932
Rotylenchus robustus ♂ apud Filipjev & Schuurmans Stekhoven 1941
Hoplolaimus uniformis Thorne 1949
Rotylenchus robustus apud Andrássy 1952
Hoplolaimus uniformis apud Oostenbrink 1954

Nec:
Anguillulina robusta ♀ apud Goodey 1932 = *R. goodeyi* nom. nov.
Anguillulina robusta apud Goodey 1940 = *R. goodeyi* nom. nov.
Rotylenchus robustus ♀ apud Filipjev & Schuurmans Stekhoven 1941 = *R. goodeyi* nom. nov.
Rotylenchus robustus apud Thorne 1949 = *R. goodeyi* nom. nov.
Rotylenchus robustus apud Goodey 1951 = *R. goodeyi* nom. nov.
Rotylenchus robustus apud Golden 1956 = *R. goodeyi* nom. nov.

Metatype: 1 Weibchen in De Man's „Hollandsche Collectie", Präparat Nr. 208, gesammelt März 1879.

Allolectotype: 1 Männchen, ebenda, Präparat Nr. 209, zur selben Zeit gesammelt.

Lokalität dieser Typen: Katwijk, Niederlande, Dünenboden.

SUMMARY

Re-examination of specimens of *Tylenchus robustus* in De Man's collection and comparison with his descriptions of this species in his papers of 1876, 1880 and 1884 have led to the conclusion that the descriptions of 1880 and 1884 were of the same species as *Tylenchus robustus* De Man 1876; further, that this species is the same as that described by Thorne 1949 as *Hoplolaimus uniformis*. As

Tylenchus robustus De Man 1876 apud De Man 1880 is the genotype of *Rotylenchus* Filipjev 1936, the specific name *uniformis* Thorne 1949 must be sunk as a synonym of it. For *Rotylenchus robustus* auctt. nec De Man, the specific name *goodeyi* is proposed.

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(*Eingegangen*: 31. Juli, 1957)

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HOPLOLAIMUS TYLENCHIFORMIS DADAY, 1905 (SYN. *H. CORONATUS* COBB, 1923) UND DIE GATTUNGEN DER UNTERFAMILIE HOPLOLAIMINAE FILIPJEV, 1936

VON

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Mit der von DADAY im Jahre 1905 aus Paraguay beschriebenen Nematoden-Art *Hoplolaimus tylenchiformis* habe ich mich schon in einem kleineren Aufsatz befasst (1954). Da DADAY's Originalbeschreibung und Abbildung ziemlich unexakt sind, erachtete ich es für notwendig, die Beschreibung der Art auf Grund des mir vorliegenden Typenexemplares in mehreren Hinsichten zu ergänzen und über das Vorderende des Tieres eine neue Abbildung zu veröffentlichen. Seitdem es bekannt geworden ist, dass ich im Besitze des ziemlich gut erhaltenen Originalpräparates von DADAY bin, wandten sich mehrere amerikanische Kollegen (G. STEINER, S. A. SHER, G. WHITTON u.a.) mit der Bitte an mich, ausführlichere Angaben über das Tier mitzuteilen bzw. die Art mit der von COBB im Jahre 1923 beschriebenen und besonders in Nordamerika ziemlich verbreiteten Art *Hoplolaimus coronatus* zu vergleichen. Diese Untersuchungen konnten aber bis jetzt nicht vorgenommen werden, weil die Art Ungarn nicht bewohnt und keine Exemplare davon mir zur Verfügung standen.

Im Jahre 1956 konnte ich endlich zum Tier gelangen, da Dr. SHER mir zwei Präparate von *H. coronatus* übersandte [1]). SHER teilte mir gleichzeitig mit, dass *H. tylenchiformis* und *H. coronatus* seines Erachtens zu derselben Art gehören. Die Präparate enthielten sehr schöne und typische Stücke der Art, und zwar 6 Weibchen und 1 Männchen. Beim Vergleich der amerikanischen Exemplare mit DADAY's Tier konnte festgestellt werden, dass sie miteinander sehr gut übereinstimmen, ja völlig identisch sind. Das DADAYsche Exemplar ist so gut erhalten, dass seine wichtigsten Eigenschaften auch heute wohl erkennbar sind. Die Scutella — die charakteristischen Merkmale der Art —

1) Für diese Freundlichkeit möchte ich Herrn SHER auch hier besten Dank sagen.

habe ich an DADAY's Tier vorher nicht wahrgenommen, jedoch nach dem Vergleich desselben mit den Exemplaren von SIER bemerkte ich, dass diese interessanten Gebilde auch dort vorhanden sind. Damit löste sich das Problem der beiden Arten: ihre Gleichheit wurde unzweifelhaft. Da nun der von DADAY gegebene Name die Priorität geniesst, muss unsere Art endgültig *Hoplolaimus tylenchiformis* Daday, 1905 (Syn. *H. coronatus* Cobb, 1923) genannt werden.

Auf Grund des Typus und der amerikanischen Exemplare teile ich im folgenden eine Neubeschreibung der Art mit.

Hoplolaimus tylenchiformis Daday, 1905
(Syn. *Hoplolaimus coronatus* Cobb, 1923)
Abb. 1-8

Masse: n = 5 ♀, L = 1,269-1,525 mm; a = 27,5-33,6; c = 55,0-86,9; V = 54,7-63,5 %. — n = 1 ♂, L = 1, 068 mm; a = 27,4; c = 90,3.

Masse des Typus nach DADAY: n = 1 ♀, L = 1,1 mm; a = 14; b = 6,7; c = ?; V = 51 %.

Kopfende abgerundet, fast halbkugelig, scharf abgesetzt. Kopfhöhe 5,5-7,0 μ, beim ♂ 7,7 μ. Kopfbreite 14,0-15,0 μ, beim ♂ 12,9 μ, letztere also zweimal so gross wie vorige. Der Kopf trägt 5-6 Kutikularinge, 2 vordere grosse, 2-3 mittlere kleine Ringe und einen hinteren grossen Ring, von denen der hintere (Basalring) durch Längsfurchen in 20-30, im allgemeinen wahrscheinlich in 24, Feldchen geteilt ist, ein für die Art sehr charakteristisches Merkmal [2]). Seitenorgan nicht nachweisbar. Kutikula stark geringelt, 1, 4-1, 7 μ dick, Ringelbreite in der Körpermitte 2,0-2,3 μ.

Die Seitenmembran, 1/4-1/6 der grössten Körperbreite, besitzt 3 Längsfelder, die in ihrer Totallänge quergestreift bzw. geringelt sind. Die beiden äusseren Felder springen etwas hervor, das mittlere sinkt also schwach ein. Die Seitenmembran beginnt kurz nach dem Kopf und ist eine Strecke lang zweireihig, da das Mittelfeld nur im Bereich des Mittelbulbus hervorkommt. Eigentümlich und typisch sind die Phasmidien. Diese leicht ovalen und stark chitinisierten, lichtbrechenden, in Obenansicht nicht porusartigen (wie bei anderen Nematoden allgemein), sondern schildförmigen Gebilde (Scutella) finden sich im

2) Die Zahl dieser Basalfeldchen ist ziemlich variierend. Beim Typus von DADAY sah ich 24 Feldchen; COBB (1923) erwähnte 32, während ihre Zahl bei den vorliegenden amerikanischen Tieren zwischen 20 und 30 schwankte.

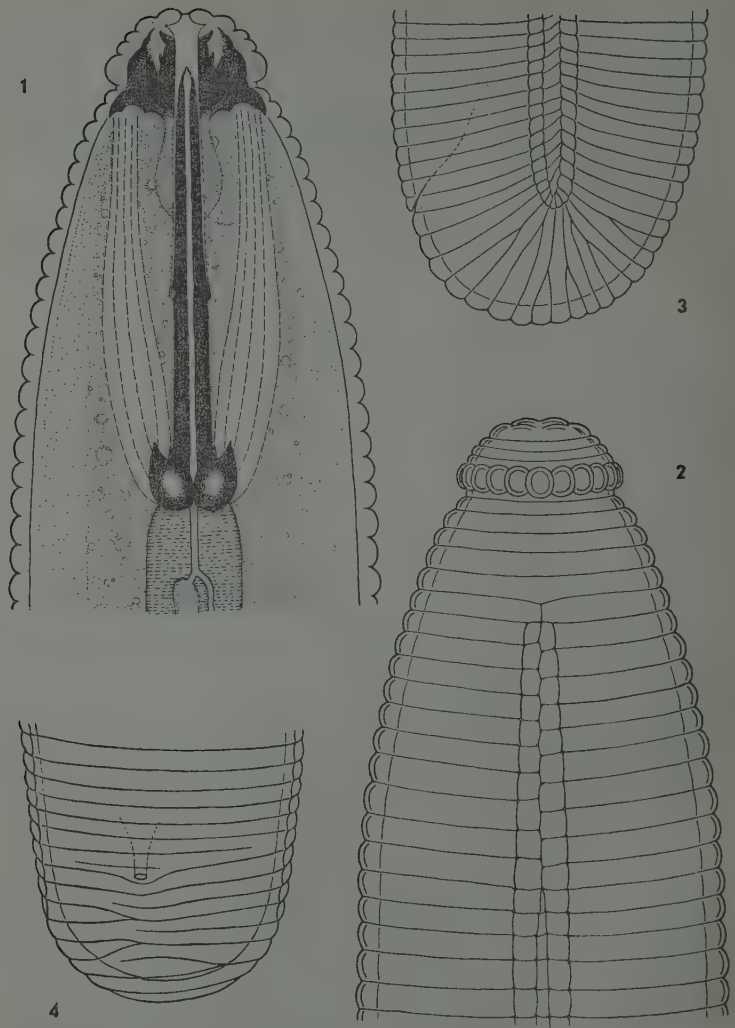

Abb. 1-4. *Hoplolaimus tylenchiformis* Daday: 1 Vorderende, innere Organisation; 2. Vorderende, Oberflächenskulptur in Lateralansicht; 3 Schwanz eines ♀, Lateralansicht; 4 Schwanz eines anderen ♀, Medialansicht.

Mittelfeld der Seitenmembranen. Ihre Länge beträgt 5,0-5,9 μ, ihre Breite 4,5-4,9 μ. Die Scutella sind je mit einer grossen, querliegenden

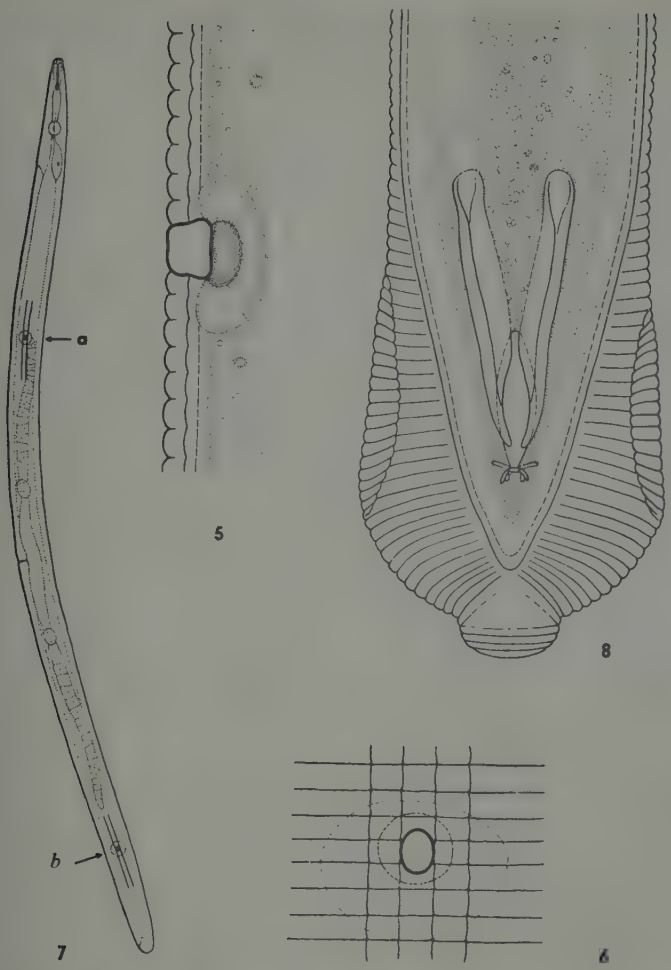

Abb. 5-8. *Hoplolaimus tylenchiformis* Daday: 5 Scutellumgegend von medial; 6 Scuttelumgegend von lateral; 7 Totalansicht eines ♀ (Scutella mit ⇄ bezeichnet; a = rechtes Scutellum, b = linkes Scutellum); 8 Hinterkörper eines ♂ in Medialansicht.

Drüse verbunden, die etwa zweimal so breit wie die Seitenmembran ist. Auch die Lage der Scutella ist sehr charakteristisch: sie liegen nämlich nie in derselben Höhe. Bei den untersuchten Weibchen war das rechtseitige Scutellum stets vor 25,6; 27.2; 27,5; 30.6; 35,7 bzw.

31,5 % beim Typus, das linkseitige aber immer hinter der Körpermitte (80,4; 82,6; 86,4; 86,5; 86,6 %) zu treffen, während beim Männchen das linkseitige vor (34,2 %), das rechtseitige aber hinter der Mitte des Körpers (84,3 %) lag [3]).

Mundstachel kräftig, 38,6-40,9 μ lang, sein Vorderteil (19,9-21,6 μ) viel stärker chitinisiert als der Hinterteil. Stachelknöpfe nach vorne zugespitzt, zweispitzig. Ein echter Führungsring fehlt. Der Ösophagus ist verhältnismässig kurz, hat einen wohlentwickelten, rundlichen Mittelbulbus mit stark chitinisiertem Lumen. Der Endbulbus fehlt, die Ösophagusdrüsen liegen frei in der Leibeshöhle. Darm mit zahlreichen Granulen bzw. Fettkörperchen, Enddarm dünn, etwa so lang wie der anale Körperdurchmesser, Analöffnung klein, queroval.

Weibliches Geschlechtsorgan paarig, symmetrisch, Ovarien gestreckt, ohne Umschlag. Vulva hinter der Körpermitte, ziemlich breit, querstehend, ihre Lippen ein wenig eingesenkt und ziemlich schwach chitinisiert. Zwischen den Ovarien und dem Uterus befindet sich je ein rundliches Receptaculum seminis enthaltend rundliche Spermien.

Spikula fast gerade, 38,6 μ lang, nach innen je mit einer membranartigen Anschwellung. Gubernakulum ziemlich gross, 19 μ, etwa halb so lang wie die Spikula, am distalen Ende mit 3 Paar kleinen Anhängen (Titillae). Bursa schwanzumfassend, breit, deutlich geringelt, ihre Ringelung aber schmäler als die des Körpers. In Medialansicht zeigt sich ein abgerundeter, kaum geringelter Zipfel am Ende der Bursa.

Schwanz des Weibchens sehr kurz, kleiner als der anale Körperdurchmesser, breit abgerundet, halbkugelig; Ringelung am Ende radiär.

Diagnose: Kutikula stark geringelt, Seitenmembran mit 3 quergestreiften Längsfeldern, Phasmidien schildförmig (Scutella), ungleich stehend, Kopf abgesetzt, am Hinterrand mit kranzartigen Platten, Mundstachel mit stärkerem Vorderteil und mit zweispitzigen Knöpfen, Ösophagusdrüsen frei in der Leibeshöhle, Darm stark granuliert, Ovarien paarig, gestreckt, Receptacula seminis vorhanden, Vulva hinter der Körpermitte, Bursa pelodär, geringelt mit Endzipfel, Spikula fast gerade, mit Innenmembranen, Gubernakulum mit Endtitillen, weiblicher Schwanz kurz, abgerundet.

Typus: 1 ♀ aus Paraguay (Daday, 1905).

Ergänzende Typen der Beschreibung: 6 ♀ und 1 ♂ aus der U.S.A. (leg. S. A. Sher).

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3) Nach Thorne (1949) befindet sich im Gegenteil das linkseitige Scutellum beim Weibchen und das rechtseitige beim Männchen vor der Körpermitte. Goodey (1951) fand im allgemeinen das rechte Scutellum vorn.

WEITERE TAXONOMISCHE PROBLEME IN DER UNTERFAMILIE HOPLOLAIMINAE

Im Jahre 1949 wurde eine andere *Hoplolaimus*-Art beschrieben, und zwar *H. uniformis* Thorne. Schon STEINER wies aber in zwei an mich gerichteten Briefen darauf hin, dass die bis dahin als selbständig erachtete Art *H. coronatus* und die Art THORNES seiner Meinung nach in dieselbe Gattung nicht einzureihen seien, da der Bau der Phasmidien der beiden Arten voneinander wesentlich abweicht. STEINER schlug vor, für die Art *coronatus* eine neue Gattung aufzustellen, bzw. nur *uniformis* und vielleicht *tylenchiformis* in der Gattung *Hoplolaimus* zu belassen. Da aber jetzt festgestellt wurde, dass auch *tylenchiformis* Scutella besitzt, ja dieses Tier und *coronatus* miteinander synonym sind, muss DADAY's Gattungsname für die Art *tylenchiformis* (syn. *coronatus*), beibehalten werden. Auch hielt ich es für richtig, THORNE's Art in eine andere Gattung einzureihen, da sie in mehreren Hinsichten (Kopfgestalt, Stachelknöpfe, Seitenmembran, Phasmidien, Gubernaku lum) von *tylenchiformis* wesentlich abweicht.

Das Problem von *uniformis* löste sich inzwischen. LOOF und OOSTENBRINK (1958) bewiesen, dass THORNE's Art mit *Rotylenchus robustus* (de Man, 1876) Filipjev, 1936 identisch ist. Da *robustus* (syn. *uniformis*) von FILIPJEV (1934 bzw. 1936) als typische Art der Gattung *Rotylenchus* bezeichnet wurde, muss die Art in derselben Gattung belassen werden. Jedenfalls wurde *uniformis* gemäss der STEINERschen Intentionen so aus der Gattung *Hoplolaimus* herausgehoben.

Der Unterfamilie Hoplolaiminae gehören also noch zwei Gattungen ausser *Hoplolaimus* an, und zwar *Rotylenchus* Filipjev, 1936 und *Helicotylenchus* Steiner, 1945. Aus der einschlägigen Literatur [4]) und auf Grund meiner Untersuchungen kann aber festgestellt werden, dass eine dieser Gattungen gleichfalls inhomogen ist. LOOF und OOSTENBRINK wiesen nämlich auch darauf hin, dass die allgemein bekannte Art *Rotylenchus robustus* in Thorne, 1949 nec de Man, 1876, (syn. *Anguillulina robusta* in Goodey, 1932 und *Rotylenchus robustus* in Goodey, 1951) mit dem Generotypus der Gattung *Rotylenchus* — *R. robustus* (de Man, 1876) Filipjev, 1936 — nicht identisch ist. Sie versahen daher diese Art zu Ehren ihres ersten Beschreibers mit dem neuen Namen *R. goodeyi* Loof & Oostenbrink, 1958. Da jetzt aber die generotypische Art *robustus* (syn. *uniformis*) von den anderen, bisher

4) Eine ausgezeichnete Übersicht der Gattungen *Rotylenchus* und *Helicotylenchus* veröffentlichte M. GOLDEN (1956).

in dieselbe Gattung eingereihten Arten in mehreren Hinsichten wesentlich abweicht, so z.B. durch die Kopfblöckchen und Titillae, würde die Gattung *Rotylenchus* Filipjev, 1936 heterogen, wenn auch diese Arten in derselben Gattung beibehalten würden. Für einen einzigen bekannten Repräsentant der Gattung *Rotylenchus* s. str. halte ich daher *R. robustus* (de Man, 1876) Filipjev, 1936.

Auch die anderen Arten von *Rotylenchus* s. lato sind in zwei, voneinander scharf abgegrenzte Gruppen zu teilen. Es gibt nämlich Arten mit normalen Phasmidien und die mit schildchenartigen Gebilden (Scutella). Da die beiden Gruppen auch durch andere Eigenschaften voneinander wesentlich abweichen, halte ich es für begründet und zweckmässig, diese Gruppen der alten Gattung *Rotylenchus* je in eine selbständige neue Gattung zu versetzen. Einen Teil der Arten — solche, die u.a. Scutella tragen (*blaberus, brachyurus, bradys, christiei* und *coheni*) — reihe ich so in die neue Gattung *Scutellonema* n. gen. ein. Für die übrigen Arten der alten Gattung (*buxophilus, goodeyi* und *pararobustus*) stelle ich eine andere neue Gattung auf, die ich nach dem hervorragenden amerikanischen Forscher, Dr. Gotthold Steiner — der meine Aufmerksamkeit auf die Hoplolaimen lenkte —, *Gottholdsteineria* n. gen. nenne.

Im folgenden wird nun eine kurze Übersicht der Genera der Unterfamilie Hoplolaiminae gegeben.

Unterfamilie Hoplolaiminae Filipjev, 1936

Tylenchidae. Kopfende mehr oder weniger abgesetzt oder vom Hals nicht abgesondert, manchmal in Feldchen geteilt. Versteifungsapparat im Kopfe kräftig, chitinisiert. Kutikula stark geringelt, Seitenmembran mit 3, oft quergestreiften Längsfeldern, ausnahmsweise rückgebildet. Deiriden fehlend. Phasmidien klein, porusartig oder ungewöhnlich gross, schildförmig (Scutella). Mundstachel wohl entwickelt, nur beim Männchen einer Art rudimentär, mit kräftigen, rundlichen oder zugespitzten Knöpfen. Mittelbulbus rundlich, Endbulbus fehlend bzw. lobus-artig, Ösophagusdrüsen frei in der Leibeshöhle; Darm stark granuliert. Vulva mehr oder weniger hinter der Körpermitte, ihre Lippen nicht chitinisiert. Weibliche Gonaden paarig, gestreckt, Receptacula seminis oft vorhanden; ovipar. Spikula tylenchoid, Gubernakulum stets deutlich, mit distaler Verdickung und manchmal mit kleinen Endfortsätzen (Titillae). Bursa meistens geringelt, schwanzumfassend, nur ausnahmsweise rudimentär. Weiblicher Schwanz kurz, stumpf abgerundet oder in einen längeren oder kürzeren Fortsatz auslaufend.

5 Gattungen: *Rotylenchus* Filipjev, 1936; *Helicotylenchus* Stei-

ner, 1945; *Gottholdsteineria* n. gen.; *Hoplolaimus* Daday, 1905 und *Scutellonema* n. gen.

Typische Gattung: *Hoplolaimus* Daday, 1905.

Schlüssel der Gattungen

1 Phasmidien von gewöhnlicher Form, klein, porusartig:
A) *Rotylenchus*-Gattungsgruppe 2
— Phasmidien auffallend gross, schildchenartig:
B) *Hoplolaimus*-Gattungsgruppe 4

2 Öffnung der dorsalen Ösophagusdrüse etwa auf $^1/_2$ Mundstachellänge hinter den Stachelknöpfen: . 2. *Helicotylenchus* Steiner
— Öffnung der dorsalen Ösophagusdrüse höchstens auf $^1/_3$ Mundstachellänge hinter den Stachelknöpfen 3

3 Kopfringelung durch Längsfurchen in Feldchen geteilt; Gubernakulum mit Titillae: . . . . . 1. *Rotylenchus* Filipjev
— Kopfringelung normal, nicht geteilt; Gubernakulum ohne Titillae: . . . . . 3. *Gottholdsteineria* n. gen.

4 Alle drei Längsfelder der Seitenmembran deutlich geringelt, bzw. Ringe rund um den Körper ununterbrochen; Stachelknöpfe nach vorne scharf zugespitzt, zweispitzig: . . 4. *Hoplolaimus* Daday
— Höchstens die beiden Aussenfelder der Seitenmembran teilweise schwach geringelt; Stachelknöpfe nach vorne abgerundet: . . . . . 5. *Scutellonema* n. gen.

A) *Rotylenchus*-Gattungsgruppe

Kopfende abgesetzt oder nicht abgesetzt. Mundstachel verhältnismässig lang und schlank. Phasmidien normal, klein, porusartig; Seitenmembran in der Gegend der Phasmidien ungeringelt.

3 Gattungen: *Rotylenchus* Filipjev, 1936; *Helicotylenchus* Steiner, 1945 und *Gottholdsteineria* n. gen.

1. Gattung: *Rotylenchus* Filipjev, 1936

Hoplolaiminae, *Rotylenchus*-Gattungsgruppe. Kopf scharf abgesetzt, Kopfringelung in zahlreiche, unregelmässige Feldchen (Blöckchen) geteilt. Entfernung zwischen den Stachelknöpfen und der Öffnung der dorsalen Ösophagusdrüse kleiner als $^1/_3$ der Stachellänge. Receptacula seminis vorhanden. Weiblicher Schwanz abgerundet, halbkugelig oder etwas eingeschnitten. Seitenmembran manchmal schwach geringelt. Gubernakulum kräftig, mit Titillae, Bursa geringelt.

1 Art:

R. robustus (de Man, 1876) Filipjev, 1936. — Synonyme: *Tylen-*

chus robustus de Man, 1876; *Tylenchorhynchus robustus* (de Man, 1876) Micoletzky, 1921 partim; *Hoplolaimus uniformis* Thorne, 1949. — Generotypus!

2. Gattung: *Helicotylenchus* Steiner, 1945

Hoplolaiminae, *Rotylenchus*-Gattungsgruppe. Kopf nur schwach oder gar nicht abgesetzt. Seitenmembran völlig glatt, ungeringelt. Entfernung zwischen den Stachelknöpfen und der Öffnung der dorsalen Ösophagusdrüse mindestens $^1/_3$ (bis $^1/_2$) so gross wie die Stachellänge. Receptacula seminis vorhanden oder fehlend. Weiblicher Schwanz abgerundet oder mit einem längeren oder kürzeren Fortsatz. Gubernakulum schlank, ohne Umschlag oder Titillae Bursa schwanzumfassend oder rudimentär, das Schwanzende nicht erreichend, mit oder ohne Ringelung.

6 Arten:

H. africanus (Micoletzky, 1915) n. comb. — Synonyme: *Tylenchus africanus* Micoletzky, 1915; *Tylenchorhynchus africanus* (Micoletzky, 1915) Schuurmans Stekhoven & Teunissen, 1938; *Rotylenchus africanus* (Micoletzky, 1915) Filipjev & Schuurmans Stekhoven, 1941.

H. erythrinae (Zimmermann, 1904) Golden, 1956. — Synonyme: *Tylenchus erythrinae* Zimmermann, 1904; *Tylenchus pseudorobustus* Steiner, 1914; *Aphelenchus dubius* var. *peruensis* Steiner, 1920; *Tylenchus spiralis* Cassidy, 1930; *Tylenchorhynchus robustus* var. *erythrinae* (Zimmermann, 1904) Bally & Reydon, 1931; *Anguillulina multicincta* in Goodey, 1932 nec Cobb, 1893; *Tylenchorhynchus multicinctus* in Schuurmans Stekhoven & Teunissen, 1938 nec Cobb, 1893; *Anguillulina erythrinae* (Zimmermann, 1904) Goodey, 1940; *Rotylenchus erythrinae* (Zimmermann, 1904) Goodey, 1951 partim.

H. iperoiguensis (Carvalho, 1956) n. comb. — Synonym: *Rotylenchus iperoiguensis* Carvalho, 1956.

H. melancholicus (Lordello, 1955) n. comb. [5]) — Synonym: *Rotylenchus melancholicus* Lordello, 1955.

H. multicinctus (Cobb, 1893) Golden, 1956. — Synonyme: *Tylenchus multicinctus* Cobb, 1893; *Rotylenchus multicinctus* (Cobb, 1893) Filipjev, 1936; *Anguillulina multicincta* (Cobb, 1893) Goodey, 1940.

H. nannus Steiner, 1945. — Generotypus!

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5) Die von Lordello als *Rotylenchus melancholicus* besprochene Art reihe ich nicht in die Gattung *Gottholdsteineria*, sondern in *Helicotylenchus* ein, da sie wegen der geringen Körpergrösse, der Schwanzform der beiden Geschlechtern, des

3. Gattung: *Gottholdsteineria* n. gen.

Hoplolaiminae, *Rotylenchus*-Gattungsgruppe. Kopf nicht abgesetzt. Seitenmembran in ihrer Totallänge glatt oder höchstens nur vorne schwach quergestreift. Entfernung zwischen den Stachelknöpfen und der Öffnung der dorsalen Ösophagusdrüse kleiner als $^1/_3$ der Stachellänge. Receptacula seminis vorhanden oder fehlend. Weiblicher Schwanz abgerundet oder ein wenig spitzig auslaufend. Gubernakulum schlank, ohne Titillae aber mit distalem Umschlag [6]).

4 Arten:

G. buxophila (Golden, 1956) n. comb. — Synonym: *Rotylenchus buxophilus* Golden, 1956.

G. goodeyi (Loof & Oostenbrink, 1958) n. comb. — Synonyme: *Tylenchorhynchus robustus* in Micoletzky, 1921 partim nec de Man, 1876; *Anguillulina robusta* in Goodey, 1932 nec de Man, 1876; *Rotylenchus robustus* in Filipjev & Schuurmans Stekhoven, 1941 und in Thorne, 1949 nec de Man, 1876; *Rotylenchus goodeyi* Loof & Oostenbrink, 1958. — Generotypus!

G. pararobusta (Schuurmans Stekhoven & Teunissen, 1938) n. comb. [7]). — Synonyme: *Tylenchorhynchus robustus* in Schuurmans Stekhoven, 1936 nec de Man, 1876; *Tylenchorhynchus pararobustus* Schuurmans Stekhoven & Teunissen, 1938; *Rotylenchus pararobustus* (Schuurmans Stekhoven & Teunissen, 1938) Filipjev & Schuurmans Stekhoven, 1941.

G. quarta Andrássy, 1958.

B) *Hoplolaimus*-Gattungsgruppe

Kopfende abgesetzt, vom Hals scharf abgesondert. Mundstachel verhältnismässig kurz, stämmig. Phasmidien sehr gross, schildchenförmig (Scutella); Seitenmembran in der Gegend der Phasmidien geringelt.

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distal nicht umgeschlagenen Gubernakulums und der rudimentären, ungeringelten Bursa eher dieser Gattung anzugehören scheint. Die Öffnung der dorsalen Ösophagusdrüse war leider bei den Typenexemplaren undeutlich.

6) Am distalen Ende, und zwar an der unteren Seite des Gubernakulums zeigt sich eine deutliche Chitinverdickung, eigentlich ein Umschlag, die bei einer Zeichnung Thorne's (1949, Abb. 2G) wohl abgebildet ist und auch bei der von mir beschriebenen Art *Gottholdsteineria quarta* ausgezeichnet erkennbar war. Dieser Umschlag scheint für die Unterfamilie charakteristisch zu sein.

7) Diese Art ist ein wenig problematisch, da die von Schuurmans Stekhoven angegebenen Masse zu schwankend sind. Es ist daher möglich, dass mehrere Arten unter dem Namen *pararobustus* zusammengezogen wurden. Das abgebildete Männchen (Schuurmans Stekhoven & Teunissen, 1938, p. 23, Fig. 9 E) weicht jedenfalls von den anderen *Gottholdsteineria*-Arten wohl ab.

2 Gattungen: *Hoplolaimus* Daday, 1905 und *Scutellonema* n. gen.

4. Gattung: *Hoplolaimus* Daday, 1905

Hoplolaiminae, *Hoplolaimus*-Gattungsgruppe. Kopf mit hinterem, kranzartigem, auf regelförmige Feldchen geteiltem Kutikularsaum und mit sehr kräftigem Versteifungsapparat. Stachelknöpfe sehr stark entwickelt, nach vorne zugespitzt, zweispitzig. Öffnung der dorsalen Ösophagusdrüse in der Nähe der Knöpfe. Alle drei Längsfelder der Seitenmembran quergeringelt oder die Seitenmembran ist in die Kutikularingelung aufgelöst. Receptacula seminis vorhanden. Weiblicher Schwanz abgerundet, Bursa geringelt, Gubernakulum kräftig mit Umschlag und Titillae. Scutella von ungleicher Lage: eins vor, das andere hinter der Körpermitte.

2 Arten [8]):

H. proporicus J. B. Goodey, 1957.

H. tylenchiformis Daday, 1905. — Synonym: *Hoplolaimus coronatus* Cobb, 1923. — Generotypus!

5. Gattung: *Scutellonema* n. gen.

Hoplolaiminae, *Hoplolaimus*-Gattungsgruppe. Kopfringelung normal, ohne in Feldchen geteilten Kutikularsaum. Versteifungsapparat schwächer. Stachelknöpfe nach vorne abgerundet, Öffnung der dorsalen Ösophagusdrüse verhältnismässig weit von den Knöpfen entfernt. Höchstens die beiden Aussenfelder der Seitenmembran schwach geringelt. Receptacula seminis meistens fehlend. Weiblicher Schwanz mehr oder weniger abgerundet, Bursa geringelt, Gubernakulum weniger entwickelt, mit distalem Umschlag bzw. dorsalem Vorsprung, ohne Titillae. Scutella fast in allen Fällen von gleicher Lage, stets hinter der Körpermitte.

6 Arten:

S. blaberum (Steiner, 1937) n. comb. — Synonym: *Rotylenchus blaberus* Steiner, 1937. — Generotypus!

S. boocki (Lordello, 1957) n. comb. — Synonym: *Rotylenchus boocki* Lordello, 1957.

S. brachyurum (Steiner, 1938) n. comb. — Synonym: *Rotylenchus brachyurus* Steiner, 1938.

S. bradys (Steiner & Le Hew, 1933) n. comb. — Synonyme: *Hop-*

8) Im Jahre 1941 wurde eine neue Art unter dem Namen *Hoplolaimus zavadskii* von Tulaganov beschrieben, zu deren Originalbeschreibung ich aber nicht gelangen konnte. So ist einstweilen ungewiss, ob die Tulaganovsche Art wirklich ein *Hoplolaimus* darstellt.

lolaimus bradys Steiner & Le Hew, 1933; *Anguillulina bradys* (Steiner & Le Hew, 1933) Goodey, 1935; *Rotylenchus bradys* (Steiner & Le Hew, 1933) Filipjev, 1936.

S. christici (Golden & Taylor, 1956) n. comb. — Synonym: *Rotylenchus christiei* Golden & Taylor, 1956.

S. coheni (J. B. Goodey, 1952) n. comb. — Synonym: *Rotylenchus coheni* J. B. Goodey, 1952.

SUMMARY

A synopsis of the genera of the subfamily Hoplolaiminae is given in the German text. *Hoplolaimus coronatus* Cobb is synonymized with *H. tylenchiformis* Daday, and the valid name of the species is therefore *Hoplolaimus tylenchiformis* Daday, 1905 (syn. *H. coronatus* Cobb, 1923). Certain species of the old genus *Rotylenchus*, which have scutellum-like phasmids, are transferred to a new genus *Scutellonema* n. gen. Similarly a new genus *Gottholdsteinema* n. gen. is established for the remaining species of the same genus, except *R. robustus* (de Man, 1876) Filipjev, 1936, which remains the single species in the genus *Rotylenchus* s. str. A key to the genera of Hoplolaiminae follows:

1 Phasmids normal, small, pore-like: . . *Rotylenchus* genus-group 2
— Phasmids extremely large, scutellum-like: . . *Hoplolaimus* genus-group 4
2 Opening of dorsal esophageal gland about ½ spear length from base of knobs of spear: . . . . . *Helicotylenchus* Steiner
— Opening of dorsal esophageal gland not more than ⅓ spear length from base of knobs of spear: 3
3 Lip region marked by annules divided into numerous small sections; gubernaculum bearing titillae: . . . . . *Rotylenchus* Filipjev
— Lip region with transverse annules only; gubernaculum without titillae: . . . . . *Gottholdsteinema* n. gen.
4 All three fields of lateral field aerolated by transverse striae; spear knobs anteriorly furcate and pointed: . . . . . *Hoplolaimus* Daday
— Center field of lateral field smooth, without transverse striae; spear knobs rounded: . . . . . *Scutellonema* n. gen.

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XIPHINEMA DE L'OUEST AFRICAIN: DESCRIPTION DE CINQ NOUVELLES ESPÈCES (NEMATODA; DORYLAIMIDAE)

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Depuis longtemps soupçonnées de parasitisme envers les végétaux, les espèces du genre *Xiphinema* Cobb 1913 sont fréquemment associées, seules ou en compagnie d'autres Nématodes, à des dépérissements de plantes variées, souvent arbustives. Ce n'est que très récemment que leur rôle parasitaire put être indirectement précisé par Adams (1955) qui, lors d'un traitement de sol de verger par un nématicide (hexachlorure benzénique), remarque le plus grande vigueur des jeunes pêchers et pommiers en même temps qu'une diminution du taux de *Xiphinema* sp. dans le sol. Il revint à Schindler (1957) et Schindler & Braun (1957) de démontrer expérimentalement les premiers le parasitisme d'une espèce de *Xiphinema*: *X. diversicaudatum* (Micoletzky 1927), Thorne 1939 sur Rosier.

Au cours de l'examen de très nombreux échantillons de sol provenant de différentes régions de l'Ouest Africain Français nous avons rencontré 7 espèces de *Xiphinema* dont 5 nouvelles:

X. hallei n. sp.
X. yapoense n. sp.
X. diversicaudatum (Micoletzky 1927), Thorne 1939
X. ebriense n. sp.
X. campinense Lordello 1951
X. setariae n. sp.
X. parasetariae n. sp.

Seuls les points de vue anatomique et systématique sont traités dans cet article. L'examen de ces espèces a permis de préciser les points suivants:

Chez toutes les espèces examinées le guide du stylet qui est dit „annulaire" s'est en fait révélé „tubulaire"; ceci est d'ailleurs déjà

visible dans certaines illustrations antérieures, notamment celles de *X. chambersi* Thorne 1939 et *X. diversicaudatum* dans l'ouvrage fondamental de Thorne (1939). Ce guide comprend un anneau basal épais, très nettement perceptible, et un tube antérieur, beaucoup plus fin, de longueur variable chez la même espèce, mais pouvant parfois atteindre 30 μ. Ce tube se rétracte d'ailleurs sous l'influence du fixateur (ici : F.A. : 4 : 10) comme l'ont montré des examens de fixations effectuées sous le microscope.

La plupart des espèces rencontrées (*X. hallei, yapoense, diversicaudatum, campinense,* et *setariae*) possèdent, aussi bien chez le mâle que chez la femelle, en plus des pores latéro-subventraux et latéro-subdorsaux, des pores ventraux régulièrement espacés. Chez *X. campinense* nous avons pu voir la connexion de ces pores avec de petites glandes ventrales sous-jacentes. L'absence ou la présence de ces pores pourraient être utilisées comme caractère systématique.

Un autre caractère utilisable serait la morphologie des cellules du cardia; celui-ci est généralement conique, large et bas, mais il peut également être étroit et haut (*X. diversicaudatum*), ou faire défaut, la base du bulbe oesophagien étant alors plane (*X. parasetariae*) ou même les cellules intestinales d'invaginant en cône dans la base de l'oesophage chez *X. setariae*; cette dernière espèce possède en outre dans la paroi oesophagienne, à 50 μ environ de la base du stylet, un petit triangle cuticularisé; ce caractère n'avait été noté que chez *X. insigne* Loos 1949.

X. ebriense n. sp. présente dans le tractus génital femelle un organe remarquable: il s'agit entre la spermathèque et l'utérus, d'un organe globuleux, très muscularisé, dont la structure n'est pas sans rappeler un bulbe oesophagien médian de *Criconematidae*; la lumière interne bordée de parois cuticularisées s'élargit au centre du bulbe; cette paroi porte à cet endroit 2 à 4 (généralement 3, mais difficilement distinguables les unes des autres par suite de l'épaisseur de la musculature) sortes de dents cuticularisées peut-être mobiles. Dans l'ignorance du rôle de cet organe (les observations vitales, très difficiles, n'ayant rien apporté) nous proposons de l'appeler „organe Z". Une telle structure ne semble avoir été décrite chez aucun *Dorylaimidae*. Chez *X. ebriense* également, les observations sur le vivant ont permis de voir qu'il existe sous les lèvres, bordant leur surface inférieure, un anneau sclérotisé très fin, capable de se retourner suivant les mouvements de sortie ou de rentrée du stylet.

Afin de permettre de mieux situer les nouvelles espèces décrites dans cet article, nous donnons ci-dessous une clé des femelles du genre *Xiphinema*. Cette clé est largement inspirée de celle publiée par Lordello (1955). Un certain nombre d'espèces a été exclu: *X. brevicaudatum* Schuurmans Stekhoven 1951, *X. effilatum* Schuurmans Stekhoven 1951 et *X. digiticaudatum* Schuurmans Stekhoven 1951 dont les descriptions sont basées sur des individus juvéniles; *X. lineum* (Grube 1849) Thorne 1939 dont la description est incomplète; *X. grande* Steiner 1914 qui d'après Thorne (1939) peut être un *Enchodelus*; *X. makrodorum* (Vanha 1893), Thorne 1939 qui représente probablement un nouveau genre; enfin *X. rotundatum* Schuurmans Stekhoven & Teunissen 1938, qui par l'absence d'ailettes à la base du stylet, le guide annulaire situé très en avant et la faible longueur du tractus génital femelle semblerait plutôt à rapporter au genre *Longidorus* Micoletzky 1922.

Clé des femelles du genre *Xiphinema* Cobb 1913

1 — Un ovaire 2
— Deux ovaires (l'antérieur régressé chez *X. krugi*) 5
2 — Queue longue (plus de 4 largeurs anales) *X. chambersi* Thorne 1939
— Queue courte (moins de 4 largeurs anales) 3
3 — Queue hémisphérique . . *X. ensiculiferum* (Cobb 1893) Thorne 1939
— Queue mamillée 4
4 — 2 paires de papilles caudales. . . . *X. brasiliense* Lordello 1951
— 3 paires de papilles caudales . . . *X. radicicola* Goodey 1936
5 — Queue longue (plus de 4 largeurs anales) 6
— Queue courte (moins de 4 largeurs anales) 7
6 — V = 29-32 % : 7 paires de papilles caudales. . *X. insigne* Loos 1949
— V = 47 % : 3-5 paires de papilles caudales . . . *X. hallei* n. sp.
7 — Queue hémisphérique ou mamillée 8
— Queue conique 13
8 — Queue hémisphérique 9
— Queue mamillée 10
9 — L = 0,8 mm. Pas d'incisure céphalique . . *X. obtusum* Thorne 1939
— L = 3 mm. Incisure céphalique marquée. . . *X. yapoense* n. sp.
10 — Lèvres ballonnées, séparées les unes des autres *X. mamillatum* Schuurmans Stekhoven & Teunissen 1938
— Lèvres lisses, soudées 11
11 — Lèvres aplaties, pas d'incisure céphalique *X. index* Thorne & Allen 1950
— Lèvres arrondies, incisure céphalique marquée 12
12 — L = 2 mm. Organe „Z" chez les ♀♀ . . . *X. ebriense* n. sp.
— L = 3-4 mm. Pas d'organe „Z" *X. diversicaudatum* (Micoletzky 1927), Thorne 1939
13 — L = au moins 8 mm. *X. cylindricaudatum* Schuurman Stekhoven & Teunissen 1938
— L = au plus 3 mm. 14
14 — V = 54 % . . . . . *X. americanum* Cobb 1913
— V = au plus 47 % 15

15 — Longueur de la queue = 1-1,2 largeurs anales; ovaire antérieur régressé; *X. krugi* Lordello 1955
— Queue plus grande que 2 largeurs anales; ovaire antérieur normal 16
16 — Tête tronquée . . . . . *X. truncatum* Thorne 1939
— Tête arrondie 17
17 — 2-3 paires de papilles caudales 18
— au moins 4 paires de papilles caudales 20
18 — 2 paires de papilles caudales 19
— 3 paires de papilles caudales . . . . . *X. pratense* Loos 1949
19 — a = 50-55; pores ventraux . . . . . *X.setariae* n. sp.
— a = 70; pas de pores ventraux . . . . *X. parasetariae* n. sp.
20 — 4 paires de papilles caudales . . . *X. campinense* Lordello 1951
— Plus de 4 paires de papilles caudales 21
21 — V = 40-42 %; 6 paires de papilles caudales. *X. elongatum* Schuurmans Stekhoven & Teunissen 1938
— V = 43-47 % . . . . . *X. italiae* Meyl 1953

Xiphinema hallei n. sp.

Pl. 1

Dimensions: ♀: L = 3,522 mm. a = 74,4 b = 8,7
c = 20,7 V = 46,7 %
♂♂: L = 3,352-3,628 mm. a = 76,7-78,1
b = 8-8,3 c = 20,8-23,8

Description:

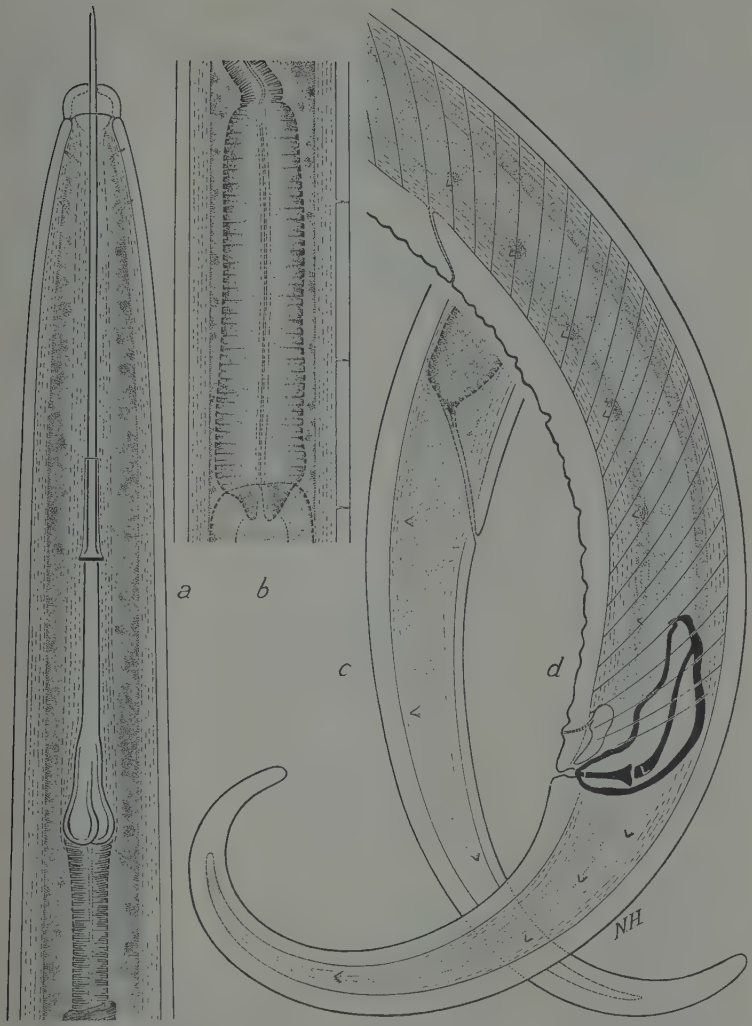
„Corps très allongé, cylindrique, peu effilé vers l'avant et très longuement vers l'arrière. Cuticule très finement striée transversalement; champs latéraux représentant $^1/_5$ du diamètre du corps. Pores latéro-subdorsaux et latéro-subventraux présents vers l'avant du corps; vers l'arrière seulement pores latéro-subdorsaux; sur tout le corps pores ventraux régulièrement espacés. Pores cervicaux supplémentaires.

Lèvres arrondies, portant deux cercles de 6 et 10 papilles. Tête séparée du corps par une incisure très nettement marquée. Ouverture des amphides au niveau de l'incisure et occupant les $^2/_3$ de la largeur.

Stylet typique du genre, long de 180-208 μ (48-51,4 % de la longueur oesophagienne), dont 71-75 μ pour la partie basale élargie (34-36 % de la longueur du stylet). Guide tubulaire de 20-22 μ de long.

Partie antérieure de l'oesophage étroite, faisant souvent une boucle avant le bulbe basal musculeux mesurant 92-96 μ de long (21,5-23 % de la longueur de l'oesophage) sur 19-21 μ. Cardia conique, large et haut.

Organes génitaux femelles: vulve située à 46,7 %, en incisure profonde, bordée de muscles très développés; vagin atteignant les $^2/_3$ de la largeur correspondante. Deux ovaires repliés, le postérieur plus développé (280 μ contre 220 μ).



Pl. 1. *Xiphinema hallei* n. sp. Mâle: a: Partie antérieure. b: Bulbe oesophagien. d: Partie postérieure. Femelle: c: Partie postérieure. (Gross. 550).

Organes génitaux mâles: deux testicules opposés, droits, souvent inégalement développés, aboutissant à un canal évacuateur en un point situé à 44-45,6 % de la longueur totale. Spicules longs de 48-50 μ,

courbés, épaissis et bosselés sur la face interne au centre, avec une petite apophyse en crochet à la partie proximale. Pièce latérale accessoire en forme de clou, de 12 μ de long. Les organes mâles supplémentaires comprennent: une paire de suppléments adanaux, à 9 μ en avant de l'anus, et 1 à 3 suppléments ventraux situés chez les ♂♂ examinés aux distances suivantes en avant de l'anus: ♂ 1: 216 μ
♂ 2: 156 μ 196 μ
♂ 3: 152 μ 180 μ 216 μ

Enfin 24 à 26 paires de papilles ventro-submédianes sont présentes.

Queue très effilée: longue de 4,2-5 largeurs anales chez le mâle, de 6,2 chez la femelle; elle présente chez le ♂ 5 paires de papilles médianes, la première au niveau de l'anus, 3 autres postérieures, la dernière à peu près à moitié de la longueur de la queue. La queue chez la femelle ne comporte que 3 paires de papilles. Chaque papille de la paire postérieure semble coiffer l'extrémité d'une sorte de canal s'élargissant vers la partie antérieure; une troisième canal se termine aveuglément près de l'extrémité de la queue; peut être s'agit-il là des vestiges des canaux déférents dépendant des glandes caudales?"

Diagnose: *X. hallei* n. sp. se différencie de *X. insigne* Loos, 1949 par les caractères suivants: taille plus grande (3,522 mm. : 1,99-2,24 mm.), vulve située plus en arrière (46,7 %: 29,8-31,6 %), stylet plus long (180-208 μ: 145-156 μ); queue, chez la femelle, n'ayant que 3 paires de papilles au lieu de 7; absence de triangle cuticularisé dans la paroi oesophagienne.

Localité type: Sol; voisinage de racines de *Drypetes mutikoro*; forêt primaire de Yapo, Côte d'Ivoire.

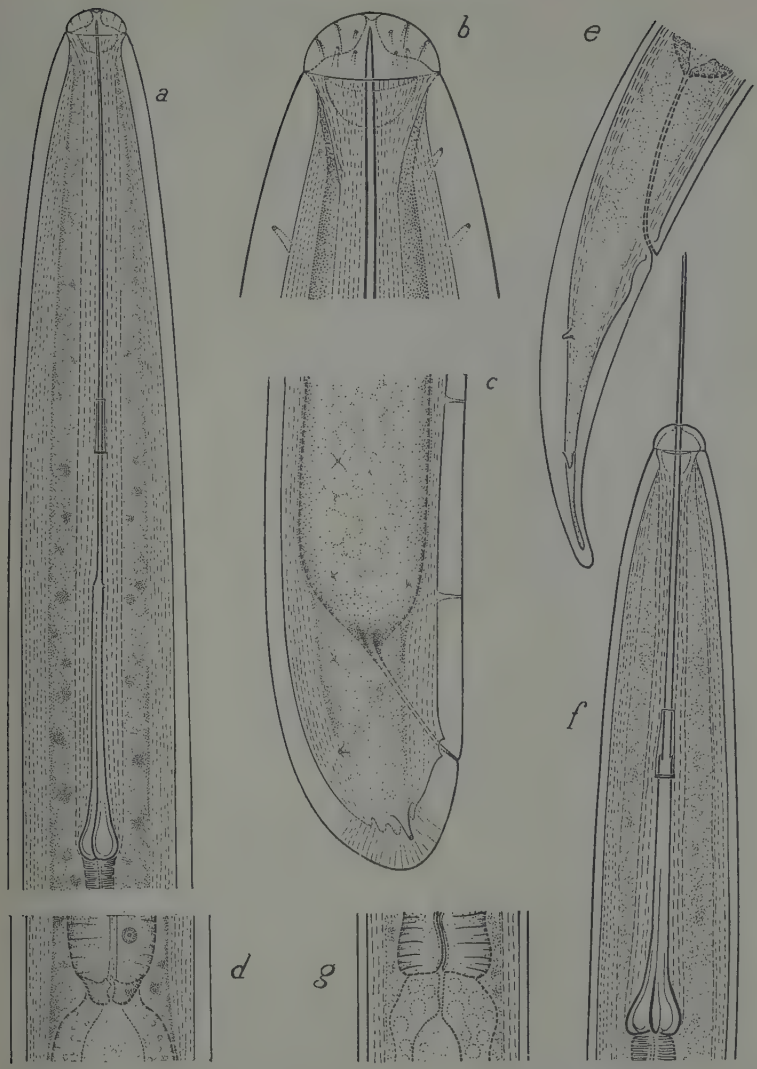
Holotype: femelle. Allotype: mâle: Tube N° 59. Laboratoire de Nématologie. I.D.E.R.T. Abidjan. Côte d'Ivoire.

Xiphinema yapoense n. sp.
(Pl. 2, a, b, c, d)

Dimensions: Femelle: L: 3,028 mm. a: 51,3 b: 6,7
c: 110,9 V: 43,9 %

Description:

„Corps cylindrique, effilé vers l'avant, très peu rétréci vers l'arrière. Cuticule épaisse, très finement striée transversalement; champ latéral occupant un quart du diamètre correspondant; pores latéro-subventraux et latéro-subdorsaux bien marqués par une sorte d'étoile sous-cuticulaire à quatre branches; ces pores deviennent submédians



Pl. 2. *Xiphinema yapoense* n. sp. Femelle: a: Partie antérieure. b: détail de la région labiale. c: partie postérieure. d: Cardia.
Xiphinema parasetariae n. sp. Femelle: f: Partie antérieure. e: partie postérieure. g: junction oesophago-intestinale.

(Gross. a, c, d, e, f, g: 550. b: 1130).

à la partie postérieure du corps; pores ventraux régulièrement espacés le long du corps; pores cervicaux présents.

Tête arrondie, lèvres non individualisées, incisure à la base de la tête fine mais nette; 2 cercles de papilles, un antérieur de 6; l'autre de 10. Amphides occupant 75 % de la largeur, au niveau de l'incisure.

Stylet typique du genre, de 209 μ de long (47 % de la longueur oesophagienne); partie basale épaissie longue de 71 μ (33,7 % de la longueur du stylet). Guide tubulaire long de 12 μ. Partie antérieure de l'oesophage étroite, sinueuse; partie postérieure renflée en un bulbe large de 16 μ et long de 87 μ (19,3 % de la longueur oesophagienne), avec deux noyaux très visibles. Cardia conique. Anneau nerveux peu développé, à 250 μ de l'avant.

Vulve en fente profonde, à 43,9 %; vagin et utérus très développés et musculairisés. Ovaires symétriques, repliés, l'antérieur plus long (780 μ contre 670 μ); au niveau de la courbure des ovaires se trouve une très vaste spermathèque remplie, sur l'individu examiné, de spermatozoïdes arrondis.

Queue subhémisphérique (longueur = 0,66 largeur anale) portant 2 paires de papilles, l'une submédiane, dans le prolongement de la ligne des pores, l'autre médiane et postérieure. Cuticule de l'extrémité caudale finement striée."

Diagnose: *X. yapoense* n. sp. diffère de *X. obtusum* Thorne 1939 par sa plus grande longueur (3 mm: 0,8 mm.) et ses lèvres hémisphériques séparées du corps par une incisure nette.

Localité type: Sol; voisinage de racines de *Drypetes mutikoro*; forêt primaire de Yapo, Côte d'Ivoire.

Holotype: Femelle; tube N° 58; laboratoire de Nématologie. I.D.E.R.T. Abidjan.

Xiphinema diversicaudatum (Micoletzky 1927), Thorne 1939.

Plusieurs dizaines de femelles appartenant à cette espèce furent trouvées au voisinage de racines de *Cinchona succirubra/ledgeriana* provenant de la Plantation de Seredou (Guinée).

Aucun mâle ne fut rencontré. Ces individus ne présentent que des différentes infimes avec la description de Thorne (1939), la plus importante étant le développement du stylet qui atteint 6-6,7 % de la longueur du corps au lieu de 4,5-4,9 %.

Xiphinema ebriense n. sp.

(Pl. 3)

Dimensions: ♀♀: L: 1,956-2,027 mm. a: 39,3-41,4
b: 5,2 -6,8 c: 45,6-61,4
V: 42,1-45,6 %
♂♂: L: 1,812-2,016 mm. a: 43,8-52,3
b: 5,1 -6,1 c: 45,3-51,2

Description:

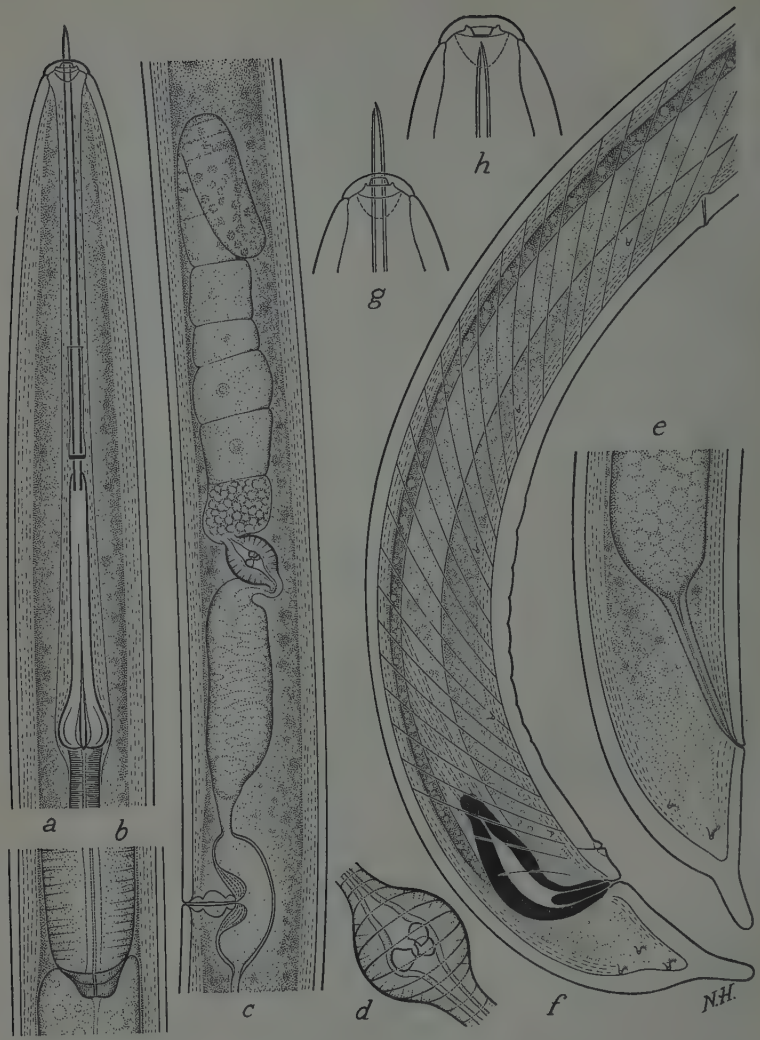
„Corps cylindrique, longuement effilé vers l'avant, très peu vers l'arrière. Cuticule très finement striée transversalement. Champ latéral très étroit vers l'avant et s'élargissant ensuite pour atteindre $^1/_3$ du diamètre du corps à la partie médiane. Pores latéraux présents, vers l'avant du corps sur une seule ligne médiane se divisant ensuite en deux.

Tête arrondie, à contours lisses, marquée par une légère incisure. Amphide au niveau de l'incisure, occupant 60-70 % de la largeur correspondante.

Stylet typique du genre, long de 171-185 μ (50,4-60,8 % de la longueur oesophagienne); partie basale épaissie longue de 62-67 μ (36-40 % de la longueur du stylet); les deux parties du stylet ne sont pas toujours situées dans le prolongement exact l'une de l'autre; les observations faites sur le vivant montrent que ce stylet possède une certaine flexibilité et lorsque l'animal se courbe, c'est la portion antérieur du stylet qui suit la courbure. Guide tubulaire assez long (jusqu'à 30 μ). A l'intérieur des lèvres, il semble exister un anneau très fin, très faiblement sclérotisé, conique, capable de se retourner lorsque saille le stylet (Pl. 3, g, h).

Oesophage à partie antérieure étroite, souvent repliée sur elle-même au contact d'un bulbe basal musculeux de 65-80 μ de long (16,4-22 % de la longueur oesophagienne) et 18-25 μ de large. Cardia conique, large et peu élevé. Intestin contenant de nombreux granules foncés. Anneau nerveux très gros, situé à peu près au milieu de l'oesophage.

Organes sexuels mâles: deux testicules droits, opposés, courts (136-276 μ), de dimensions à peu près égales; partie spermogoniale courte, n'occupant que le $^1/_3$ de la longueur du testicule; junction des deux testicules dans le canal déférent à 42-46 %. Deux spicules arqués en faucille, à partie médiane épaissie, longs de 39-45 μ. Pièce latérale droite, courte, simple. Suppléments sexuels: une paire de suppléments adanaux, une papille préanale à 203-268 μ de l'avant, 5 paires de papilles subventrales.



Pl. 3. *Xiphinema ebriense* n. sp.: Femelle: a: partie antérieure. b: cardia. c: tractus génital. d. „organe Z". g, h: détail de la région labiale. e: partie postérieure. Mâle: f: partie postérieure.

(Gross.: a, b, c, e, f: 550. d, g, h: schématique).

Organes sexuels femelles: le tractus génital femelle comprend les organes suivants: l'ovaire, une spermathèque peu développée, un orga-

ne énigmatique (nommé „organe Z'"): il s'agit d'un bulbe sphérique, constitué de muscles circulaires puissants; la lumière, bordée de parois sclérotisées, se trouve en connexion avec d'un côté la spermathèque de l'autre l'utérus; cette lumière est élargie au centre du bulbe où se trouvent 2 à 4 (le plus souvent 3) apophyses cuticularisées, très réfringentes, attachées à la paroi par une partie arrondie et présentant deux pointes vers l'extrémité distale, ressemblant en quelque sorte à des dents mobiles; la signification et le rôle de cet organe, retrouvé chez toutes les femelles examinées, nous échappent. Utérus vaste à paroi faisant de nombreux plis. Vulve en fente équatoriale, à 42,1-45,6 %, musculature sous-jacente divisée en 4 secteurs.

Queue mamillée; partie rétrécie faisant environ $^1/_3$ de la longueur totale; celle-ci équivalent à 1,1-1,3 largeurs anales. La queue porte 3 paires de papilles chez le mâle; chez la femelle 2 seulement sont le plus souvent visibles."

Diagnose: les femelles de l'espèce décrite se différencient de toutes celles décrites par la présence de l'organe Z. En outre *X. ebriense* n. sp. se différencie par ses lèvres soudées et hémisphériques, à suture céphalique nette, de *X. mamillatum* Schuurmans Stekhoven & Teunissen 1938 (lèvres ballonnées et détachées les unes des autres) et de *X. index* Thorne & Allen 1950 (lèvres aplaties, par de suture céphalique visible). Sa faible taille (1,8-2,0 mm.) l'oppose à *X. index* (3,4-3,6 mm.) et *X. diversicaudatum* (Micoletzky 1927) Thorne 1939 (4,0-4,3 mm.); de même la composition des suppléments sexuels chez le mâle: 1 paire de suppléments adanaux, 1 seul supplément ventral et 5 paires de papilles ventro-submédianes; *X. index*: 1 paire de suppléments adanaux, 4 suppléments ventraux, 1 organe innervé antérieur, 7 paires de papilles submédianes. *X. diversicaudatum*: 1 paire de suppléments adanaux, 3-4 suppléments ventraux, pas de papilles ventro-submédianes signalées. De plus cette espèce ne comporte que 2 paires de papilles caudales chez le mâle alors que *X. ebriense* en comporte 3.

Lieu type: Sol; voisinage de racines de *Coffea canephora* atteint de trachéomycose à *Fusarium xylarioides*; Adiopodoumé. Côte d'Ivoire.

Holotype. Femelle. Tube A 19. Laboratoire de Nématologie. I.D.E.R.T. Abidjan. Côte d'Ivoire.

Allotype. Mâle. Tube A 18. Laboratoire de Nématologie. I.D.E.R.T. Abidjan. Côte d'Ivoire.

Xiphinema campinense Lordello 1951
(Pl. 4 a, b)

Dimension: L: 2,503-2,814 mm. a: 51,8-55,1
b: 6,1 -8,5 c: 29,4-37,8
V: 40,5-42 %

Description:

Corps cylindrique, peu effilé vers l'avant, un peu plus vers l'arrière. Cuticule non striée. Pores latéro-subdorsaux présents tout le long du corps, les latéro-subventraux à la partie postérieure seulement et sur une longueur très variable suivant les individus. Pores ventraux, correspondant à des glandes ventrales, plus discrets mais présents; pores cervicaux présents. Champ latéral occupant 26 % du diamètre correspondant.

Tête arrondie séparée du corps par une incisure nette, lèvres soudées; amphides larges: 90 % de la largeur correspondante, situées au niveau de l'incisure.

Stylet typique du genre, long de 185-193 μ (53,4 % de la longueur oesophagienne) partie basale élargie longue de 70,5-75 μ (37-40 % de la longeur totale du stylet). Guide tubulaire long de 11-28 μ.

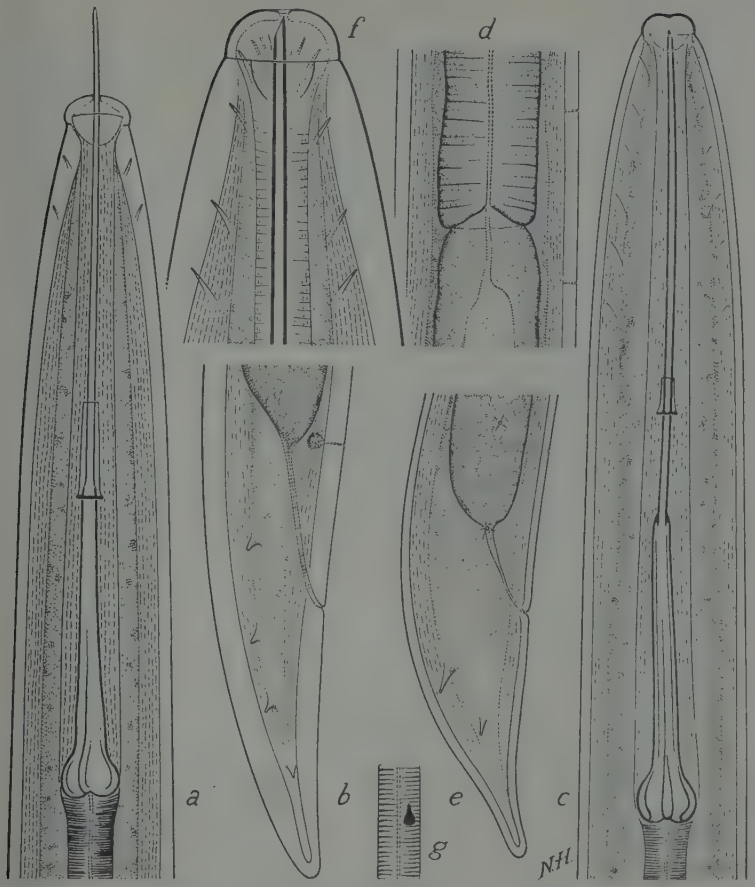
Partie antérieure de l'oesophage étroite, faisant souvent une boucle avant le bulbe postérieur de 83-97 μ de long sur 23-27 μ de large; quelquefois même la partie antérieure est repliée au point que le bulbe basal vient au contact direct du stylet. Cardia peu développé, obscur.

Organes génitaux femelles: Vulve en croix, à 40,5-42 %. Ovaires repliés, sans caractères spéciaux, Longueur totale de l'ovaire antérieur: 208-308 μ; de l'ovaire postérieur: 208-314 μ.

Queue effilée, régulièrement conique ou présentant parfois un léger rétrécissement subterminal; longueur égale à 2,6-3,2 largeurs anales. La queue porte 4 paires de papilles, la postérieure latérale, les trois autres latéro-subdorsales: la paire antérieure est souvent située un peu au-dessus de l'anus.

Taxonomie: les caractères des individus décrits ci-dessus s'accordent avec ceux donnés par Lordello pour *X. campinense* dans sa description originale (1951) et avec les précisions apportées ultérieurement (Lordello et Arruda, 1956); seul diffère le stylet légèrement plus court (154-161 μ: 185-193 μ).

Lieu de récolte: Sol; voisinage de racines de *Cocos nucifera*; plusieurs localités du Togo Français.



Pl. 4. *Xiphinema campinense* Lordello 1951. Femelle: a: partie antérieure. b: partie postérieure. *Xiphinema setariae* n. sp.: Femelle: c: partie antérieure. d: junction oesophago-intestinale. e: partie postérieure. f: détail de la région céphalique. g: triangle cuticularisé dans la paroi oesophagienne.
(Gross: a, b, c, d, e: 550. f.: 1130. g: schématique)

Xiphinema setariae n. sp.

(Pl. 4 c, d, e, f, g).

Dimensions: Femelles; L: 2,664-2,930 — a: 49,8-55,2
b: 6,0 -6,9 — c: 41,7-46,7
V: 35,4-38,5 %

Description:

„Corps cylindrique aminci aux deux extrémités. Cuticule très finement striée transversalement (stries = 0,6 μ au milieu du corps). Champ latéral embrassant environ $^1/_3$ du diamètre du corps. Pores latéro-subventraux présents sur tout le corps; pores latéro-subdorsaux uniquement dans la partie postérieure; pores ventraux et pores cervicaux supplémentaires présents également.

Région labiale arrondie, séparée du reste du corps par une incisure marquée. Amphides correspondante à 75 % de la largeur, au niveau de l'incisure.

Stylet typique du genre, long de 192-199 μ (41,4-47,5 % de la longueur oesophagienne); partie basale élargie longue de 72-77 μ (36-40 % de la longueur totale du stylet). Guide tubulaire de 10-21 μ. Partie antérieure de l'oesophage étroite; Bulbe basal allongé, de 116-132 μ (24-30 % de la longueur oesophagienne) sur 23-29 μ de large. A 43-56 μ de la base du stylet existe dans la paroi oesophagienne une petite structure cuticularisée en triangle, de 3 μ de long sur 1,5 μ de large. Pas de cardia visible, les cellules intestinales au contraire, sont invaginées dans le bulbe oesophagien. Anneau nerveux gros. Intestin rempli de granules sombres.

Vulve en croix, à larges branches latérales. Deux ovaires repliés, le postérieur plus développé; longueur totale de l'ovaire antérieur: 365-604 μ; ovaire postérieur 772-840 μ. Oeufs de 140 × 35 μ. Jamais plus d'un oeuf complètement développé par ovaire, et un seul ovaire ayant 1 oeuf à la fois.

Queue conique à extrémité digitée; longueur de la queue: 2-2,3 largeurs anales. Deux paires de papilles caudales. Cuticule de l'extrémité finement fibrillée."

Diagnose: *X. setariae* n. sp. se différencie des autres espèces décrites par l'absence de cardia conjugée à la présence d'un triangle cuticularisé dans la paroi oesophagienne; elle se sépare de *X. pratense* Loos 1949 par: sa taille plus grande (2,7-2,9: 2,02-2,13 mm), la présence d'un fragment cuticularisé dans la paroi oesophagienne, la présence de 2 paires de papilles caudales au lieu de 3.

Elle se différencie de *X. campinense* Lordello 1951 par ses deux paires de papilles caudales au lieu de 4 et la forme de sa queue.

Localité type: Sol, voisinage des racines de *Setaria megaphylla*. Adiopodoumé. Côte d'Ivoire.

Holotype. Femelle. Tube N° 84. Laboratoire de Nématologie. I.D.E.R.T. Abidjan. Côte d'Ivoire.

Xiphinema parasetariae n. sp.
(Pl. 2 e, f, g).

Dimensions: Femelle: L: 2,944 mm. a: 70,1 b: 7,4
c: 36,3 V: 40,2 %

Description:

„Corps cylindrique, effilé, aminci aux deux extrémités. Cuticule non striée. Champ latéral représentant un peu moins du tiers du diamètre correspondant. Pores latéro-subventraux et latéro-subdorsaux présents. Pas de pores ventraux.

Région labiale subhémisphérique, séparée du corps par une incisure nette. Amphide occupant presque les $^2/_3$ de la largeur, au niveau de l'incisure.

Stylet typique du genre; long de 187 μ (47 % de la longueur oesophagienne); partie basale élargie représentant 36,5 % de la longueur du stylet. Guide annulaire long de 26 μ.

Partie antérieure de l'oesophage étroite, formant parfois une boucle; bulbe postérieur long de 98 μ (24,5 % de la longueur oesophagienne) et large de 21 μ. Pas de cardia marqué. Anneau nerveux moyen, à 271 μ de l'avant.

Organes génitaux femelles: vulve en fente profonde, à 40,2 %. Deux ovaires repliés, sans caractères spéciaux. Ovaire antérieur: 210 μ; ovaire postérieur: 234 μ.

Queue conique, allongée (longueur: 3,2 largeurs anales), extrémité arrondie. Deux paires de papilles caudales."

Diagnose: espèce voisine de *X. setariae* n. sp., s'en différenciant par son corps plus élancé (a = 70: 50-55), sa queue plus effilée, l'absence de striation cuticulaires, de pores ventraux et de triangle cuticularisé dans la paroi oesophagienne.

Localité type: Sol, voisinage de racines de *Oryza sativa*. Kankan. Guinée Française.

Holotype: Femelle: Tube A 122. Laboratoire de Nématologie. I.D.E.R.T. Abidjan. Côte d'Ivoire.

SUMMARY

The author describes and figures five new species of *Xiphinema* from French West Africa: *X. hallei*, *X. yapoense*, *X. ebriense*, *X. setariae* and *X. parasetariae*. Details are given of the anatomy of these species and of *X. campinense* Lordello 1951. The so-called "guiding ring" of the spear is in reality a "guiding tube". In addition to latero-subventral and latero-subdorsal pores, ventral pores are present in nearly all the species examined. *X. ebriense* possesses in the female gonad a very enigmatic muscular structure called "organe Z". The presence of *X. diversicaudatum* (Micoletzky 1927), Thorne 1939 is also noted.

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(*Accepté: le 23 Août 1957*)

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SEVERE EELWORM (*DITYLENCHUS DIPSACI* (KÜHN) FILIPJEV) INFESTATION OF THE NARCISSUS VARIETY SOLEIL D'OR

BY

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The growing of early daffodils is a highly important industry on the Isles of Scilly. Ground suitable for cultivating this valuable crop is scarce and, due to the uneconomical nature of alternative crops, a close rotation of narcissus is frequently practised. Soleil D'Or has long been established as one of the most successful varieties because it provides a major contribution to the early crop.

During 1954, several instances of premature, slimy decay were noticed in this variety. Specimens examined revealed very heavy infestations of stem and bulb eelworm, *Ditylenchus dipsaci* (Kühn) Filipjev.

Symptoms of Severe Infestation

Typical eelworm infestation of narcissus produces a number of symptoms. Spickels appear in the young foliage and, at a later stage, arrested growth may cause stunting and distortion. The flower stem may be similarly affected so that an inferior flower or blindness results. When an infected bulb is cut across it shows brown rings of dead and dying scales. Heavily infested dormant bulbs are often soft to the touch and easily disintegrate.

Symptoms of severe eelworm infestation in Soleil D'Or exhibit additional features illustrated in Figure 1. Spickels, regarded by Southey (in litt.) as a sensitive indication of eelworm presence, are usually the earliest sign of attack. However, as eelworms increase in the growing foliage they cause a more widespread disruption of cellular tissue and the discrete areas of damage demarcated by the spickels lose their definition. Further increase in numbers of eelworms causes leaves to become pallid with yellowish mottling and longitudinal streaking. Flowering is delayed and at the same time curvature of the

foliage and distortion of the flower stem with general stunting may be pronounced. Later the foliage becomes swollen, easily torn and is frequently sloughed off. Finally the presence of a heavy eelworm burden causes leaves to decay prematurely. Brownish slimy areas in the leaves enlarge until they are consumed in a general necrosis which is most advanced near the neck of the bulb.

The dormant bulb from a heavily infested plant often exhibits a hollow neck due to the die back of the previous year's foliage. In Soleil D'Or, brown rings are rarely present in the bulb during the growing period or in dormancy and generally the bulb of this variety is a poor indicator of eelworm presence. Very often the bulb of an otherwise heavily infested plant shows no sign of eelworm attack. Eelworms may cause diffuse areas of brownish decay but rarely is a scale entirely damaged so as to form a brown ring. The bulb of this particular variety seems to inhibit development of *D. dipsaci* and the eelworms are almost completely restricted to the centre of the bulb.

Staniland (1932) recorded four cases of unusual eelworm attack on Soleil D'Or from Cornwall and East Devon. The flower stem was deformed so that the upper part bent downwards like the crook of a walking stick; eelworms were especially numerous in this 'crooked' portion and in the foliage. "Later in the season the flower stems died down very rapidly, being reduced to a rotting mass and then eelworms were exceptionally numerous; saprophytic eelworms were also found in large numbers. Later still, when all the foliage had withered, a very large concentration of eelworms was found in the centre of the bulb, but only in the area around the flower stem". Referring to the bulbs, Staniland mentioned that only a very small percentage showed typical rings indicating the presence of eelworm and in no case was the amount of ringing severe.

Pattern of Eelworm Distribution

In order to correlate the distribution of *D. dipsaci* with the symptoms described, samples of four or five grams were taken from four regions of the plant; bulb base, bulb neck, lower foliage and upper foliage. Eelworms were extracted from the chopped plant tissues in Baermann funnels and counted by a dilution technique.

From Table I it is evident that the most eelworms occur in the foliage of Soleil D'Or and in the growing plant their number increases enormously from bulb base to upper foliage. These quantitative differences are compared with four infested plants of King Alfred, two

A B C

Fig. 1. Severe eelworm infestation in the narcissus variety Soleil D'Or. A and B, early and late stages of attack. C, plant dissected to show slimy necrosis of the foliage and damage to the centre of the bulb.

of which possessed well defined spickels whilst the other two had intermediate leaf symptoms indicating a higher eelworm infestation.

TABLE I

Counts of D. dipsaci per grm. of Tissue

Bulb	Variety	Symptoms on Foliage	Bulb Base	Bulb Neck	Lower Foliage	Upper Foliage
1	Soleil D'Or	Severe	680	2390	14160	29300
2	" "	"	360	2460	4870	6850
3	" "	"	180	275	1902	4768
4	" "	"	24	50	1409	3833
5	King Alfred	Intermediate	579	278	1651	1014
6	" "	"	586	21	285	102
7	King Alfred	Slight	0	0.4	3.6	32
8	" "	"	0.6	0.8	0.6	3.4

EELWORM DISTRIBUTION IN THE BULB

The major part of a narcissus bulb is formed of scale leaves which surround a varying number of scale-like sheathing leaves protecting the true "foliage" leaves and flower bud (CHAN 1951). After dissecting two bulbs into these components, horizontal borings 8 mm. in diameter were taken with a cork borer through each.

There were no brown rings in either bulb and, as shown in Table II, *D. dipsaci* was practically confined to the region of the "foliage" leaves near the centre of the bulb.

TABLE II

Recovery of D. dipsaci from two bulbs of Soleil D'Or

No. complete Scales from which Cores Taken			Wt. of Cores in gms.		Eelworm Count per Core	
Location	Bulb 1	Bulb 2	Bulb 1	Bulb 2	Bulb 1	Bulb 2
Scale leaf outer	3	2	.2500	.1278	0	6
Scale leaf inner	3	2	.2564	.1519	0	48
Sheathing scale-like leaf	2	2	.0906	.0789	0	5
"Foliage" leaf	4	4	.1634	.1449	427	10,720

Inoculation Tests

Eelworms from Soleil D'Or were used to reproduce infestations in this and other varieties. A stock of Isles of Scilly bulbs was collected during the summer of 1956, and hot water treated prior to planting outdoors at Starcross. Five bulbs of ten varieties were planted individually in ash plunged pots. Approximately 500 eelworms were added to the first pot in each variety, 1,000 to the second, 3,000 to the third and 4,000 to the fourth; the fifth remained as a control.

Symptoms of foliage infestations were first noticed thirteen weeks later and all the varieties except Scilly White showed symptoms of various sorts by the fifteenth week. Later, when plants were lifted for eelworm counts, this reputedly resistant variety possessed a few small spickels on the lower foliage surrounded by the sheathing scales. Slimy necrosis of the inner foliage in Soleil D'Or occurred in the nineteenth week after inoculation, and shortly afterwards Magnificence and Verger developed spongy, easily torn foliage. Assessing susceptibility on foliage symptoms, the last two varieties seemed to come next in order to Soleil D'Or; spickels on them tended to coalesce in longitudinal yellowish ribs whilst the foliage and flowers were very stunted. Eelworm infestation appeared to cause a delay in flowering, since in all the varieties recorded, it was the control plant which flowered first.

The two rows of plants receiving approximately 4,000 and 3,000 eelworms per pot were lifted 20 and 23 weeks after inoculation. Eelworms were recovered from all the foliage and bulb tissue of each plant.

Recovery of eelworms is illustrated in Figure 2. *D. dipsaci* caused severe infestation in the highly susceptible variety Soleil D'Or and varying degrees of infestation in the nine other varieties. There was at least a tenfold increase of eelworms in Soleil D'Or during the first 23 weeks of its growth after receiving 3,000 eelworms.

Eelworm symptoms expressed in narcissus foliage are probably an indication of the infestation in that part of the plant only. Figure 2 shows that bulb and foliage differ in their susceptibility to eelworm invasion from one variety to another. Excepting Verger, these results also suggest that for any one variety a constant relationship exists between the degree of bulb and foliage penetration. Soleil D'Or is an example having highly susceptible foliage and a "resistant" bulb whereas Helios, Fortune, Carlton and The First appear to have foliage which is relatively more resistant than the bulb. Thus the presence of clearly defined spickels appears to be associated with resistant foliage and may

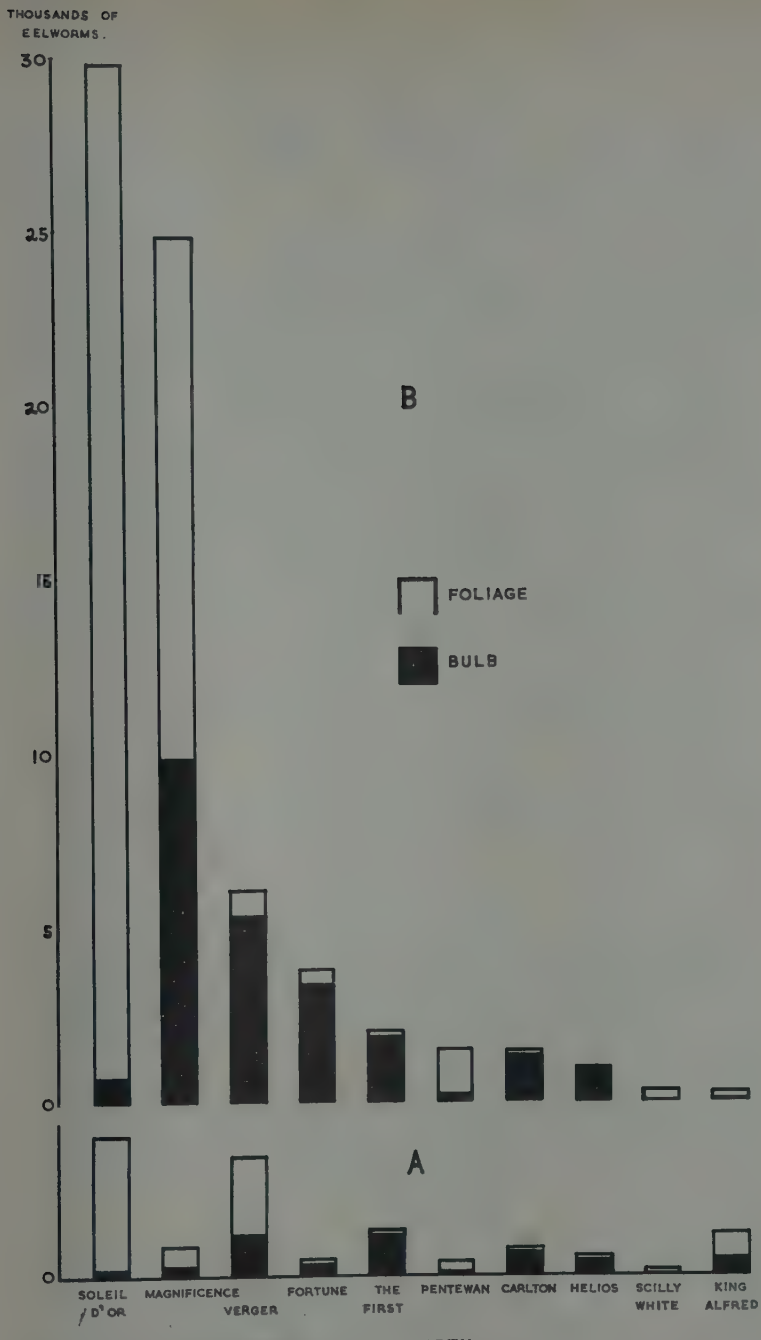

Fig. 2. Bulb inoculation tests. Total recovery of eelworms from ten varieties of narcissus inoculated with *D. dipsaci* from Soleil D'Or. A and B. 20 and 23 weeks after each bulb received 4,000 and 3,000 *D. dipsaci* respectively.

indicate a relatively high infestation in the bulb. Conversely, brown rings in the bulb would not necessarily mean that there was also a heavy infestation in the leaves.

DISCUSSION

Much attention has been paid to the successful control of eelworms in narcissus by hot water treatment of the bulb. In severe infestations the high populations built up in the foliage and disseminated in the soil should be borne in mind where close bulb rotations are practised.

Soleil D'Or flowers from late November to March on the Isles of Scilly at a time when its resistance to eelworm attack is low. Reinfestation of foliage may occur from eelworms in the soil but the central reservoir of eelworms in Soleil D'Or bulbs probably constitutes the more important source. Under these conditions a heavy eelworm burden could develop rapidly in the favourable environment of the foliage.

In searching for eelworm infested narcissus plants, growers rely mainly on feeling spickels in the foliage or cutting a bulb across to look for brown rings. However, both these features may be absent in severe infestations and, considering the different symptoms expressed by varieties of narcissus, any arrested growth should be regarded with suspicion.

ACKNOWLEDGMENTS

I wish to thank W. J. Moyse and P. Wiggell for their help in field work on the Isles of Scilly and H. C. Woodville and W. F. Buck for the photographs.

ZUSAMMENFASSUNG

Die Narzissen-Abart Soleil d'Or erwies sich gegen *Ditylenchus dipsaci* höchst anfällig. Es bestand ein regelmässiges Bild der Nematodenverteilung bei den stark befallenen Pflanzen, wobei die Zahl der Nematoden sich von den unteren Teilen der Zwiebel angefangen bis zum obersten Blätterwerk erhöhte. Innerhalb der Zwiebel waren die Nematoden praktisch auf die inneren Schalen beschränkt.

Symptome starken Ausmasses ergaben sich bei Soleil d'Or während der Impfversuche. Innerhalb von 23 Wochen nach der Impfung nahmen die Nematoden bei dieser Abart 10-fach zu. Neun andere Narzissen-Abarten, die mit Nematoden von Soleil d'Or geimpft worden waren, zeigten Blattschaden verschiedenen Grades, die durch Nematodenzählungen belegt wurden.

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(*Received for publication*: September 4, 1957.)

SCIENTIFIC NOTES

Hermann GISIN, *Formation of an International Group for the Study of the Fauna of the Soil*[1]).

During the Sixth International Congress of Soil Science at Paris in 1956, zoologists specializing in the study of the fauna of the soil formed an international group with the object of providing a means for the exchange of information amongst workers in this field. To achieve this object, a news bulletin is being published, and in addition international colloquia on particular aspects of the subject will be held at intervals.

The second number of the Bulletin was published in October, and includes a questionnaire requesting details of current research projects together with information of animal groups studied. The results of this enquiry will be published in future numbers of the Bulletin. Anyone interested in this field of research, and who has not received the Bulletin may obtain a copy by writing to: — Mr. J. D'AGUILAR, Station centrale de Zoologie agricole, Route de St-Cyr, Versailles, France.

The first colloquium devoted to: 'Methods of Research in Soil Zoology' will be held at Rothamsted Experimental Station, Harpenden, Herts., England, from the 10th to 14th July, 1958, immediately prior to the International Zoological Congress to be held in London. The next number of the News Bulletin will give the programme and other details of the Colloquium.

A. C. TARJAN, *Note on the selection of types for certain nematode species*[2]).

In conformance with the International Rules of Zoological Nomenclature, it is desired to establish *Hemicycliophora parvana* Tarjan, 1952, and *Tylencholaimus brevicaudatus* (Tarjan, 1953) Tarjan, 1956, on an objective basis. The lectotype of *H. parvana* is mounted in glycerin and is designated Slide 18, Tray 1, Cabinet 2724 in the Nematode Collection, Citrus Experiment Station, Lake Alfred, Florida. The lectotype of *T. brevicaudatus* is similarly mounted on Slide 15, Tray 1, Cabinet 2724 of the same collection.

Additional descriptive information on *Xiphinema americanum* Cobb, 1913, was published by the author in 1956. It is believed that COBB's original type material of this species was not saved. This has been corroborated by A. L. TAYLOR, Principal Nematologist, Plant Industry Station, Beltsville, Maryland, and G. THORNE, Professor of Plant Pathology and Zoology, University of Wisconsin, Madison. In his description of *X. americanum*, COBB (1913) gave the habitat and distribution as "About the roots of a variety of plants,—corn, grass, citrus trees, on the Atlantic and Pacific Slopes of the United States". The neotype selected for this species is a male mounted in glycerine on Slide 16, Tray 1, Cabinet 2724 in the above mentioned collection. It was collected by the author on October 10, 1952, from roots of bent grass (*Agrostis* sp.) and surrounding soil, directly north of the Agronomy Department Turf Shed, University of Rhode Island, Kingston. This neotype already has been figured and described (TARJAN, 1956) and is consistent with COBB's original description.

In a recent paper (TARJAN, 1957), the neotype of *Ecphyadophora tenuissima* de Man, 1921, was designated solely on information that DE MAN's "Algemene

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1) Muséum de Genève, President of the Zoology Committee (Biology Commission) of the International Society of Soil Science.

2) Citrus Experiment Station, Lake Alfred, Fla., U.S.A.; Florida Agricultural Experiment Station Journal Series No. 686.

collectie I-IV" did not contain the single specimen of this species on which his original description was based. A letter from Dr. M. Oostenbrink, Plantenziektenkundige Dienst, Wageningen, Holland, likewise establishes the absence of this specimen from the "Hollandische collectie de Man I, II", thereby further justifying the designation of the neotype.

Cobb, N. A. (1913). New nematode genera found inhabiting fresh water and non-brackish soils. — *J. Wash. Acad. Sci.* **3**: 432-444.

de Man, J. G. (1921). Nouvelles recherches sur les nematodes libres terricoles de la Hollande. — *Capita zool.* **1**: 1-62.

Tarjan, A. C. (1952). The nematode genus Hemicycliophora de Man, 1921 (Criconematidae) with a description of a new plant-parasitic species. — *Proc. helm. Soc. Wash.* **19**: 65-77.

—— (1953). Known and suspected plant-parasitic nematodes of Rhode Island, I. — *Proc. helm. Soc. Wash.* **20**: 49-54.

—— (1956). Known and suspected plant-parasitic nematodes of Rhode Island II. *Xiphinema americanum* with notes on *Tylencholaimus brevicaudatus* n. comb. — *Proc. helm. Soc. Wash.* **23**: 88-92.

—— (1957). Observations on Ecphyadophora tenuissima de Man, 1921. — *Nematologica* **2**: 152-158.

A checklist of soil and plant-parasitic nematode species has been compiled over the past three years and is rapidly nearing completion. This work was undertaken in full realization that the science of plant nematology has been plagued by the confusion that surrounds the correct nomenclature of certain nematode species. Some species names are incorrectly cited in scientific papers due to the inaccessibility to workers of literature dealing with the taxonomic revisions these species have undergone. Other species, in past decades, have been erroneously cited and these errors have been perpetrated. Still others have been named and have been overlooked or disregarded so that it is unknown what the generic or taxonomic status of those species currently is.

The checklist deals mainly with terrestrial and plant nematodes. Species in purely aquatic or animal-parasitic genera have been disregarded unless sometime, in the nomenclatorial history of the species, it was transferred to a genus we recognize as being a soil form. The species of three genera have been recorded almost completely, *Monhystera, Rhabditis,* and *Aphelenchus.* All of the species in the new genera resulting from the recent splitting of *Rhabditis* and *Aphelenchus* likewise are presented.

Under each specific name (subspecific and intrasubspecific names are included) arranged in alphabetical order will be listed all generic and subgeneric changes, synonymies, homonymies, the current nomenclatorial status, and the authority for each of the listed changes.

The work which is about 95 percent complete should be ready within two years and tentatively is planned for release as a Florida Agricultural Experiment Station publication. The many problems encountered in attempting to assemble this checklist in an unbiased but accurate manner have, for the most part, been resolved by adhering to a strict interpretation of the International Rules of Zoological Nomenclature.

In an undertaking of this nature, it is necessary personally to scrutinize many of the original descriptions and taxonomic changes which have appeared in the literature. It would be greatly appreciated if anyone, not yet directly contacted, would send reprints dealing with nematode taxonomy to

A. C. Tarjan, Citrus Experiment Station, Lake Alfred, Florida, U.S.A.

Nematologica III (1958): 81-90. E. J. Brill, Leiden

DAY LENGTH AND CYST FORMATION IN THE POTATO ROOT EELWORM, *HETERODERA ROSTOCHIENSIS* WOLLENWEBER

BY

C. ELLENBY

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In a previous communication (ELLENBY, 1946) a comparison was made between the cysts formed on a number of different varieties of the potato, *Solanum tuberosum* L. It was shown that the cysts formed on Doon Star plants were smaller than those formed either on Redskin or Arran Banner plants, that, even when differences in cyst size were allowed for, they contained fewer eggs, and that even when differences in egg number were allowed for, they produced fewer larvae. The results therefore confirmed, and, in certain respects, extended those reported by GEMMELL (1943), who working with Doon Star, Epicure, Golden Wonder, and Majestic, found that the first two varieties bore fewer and smaller cysts than those formed on the latter two, and that these cysts also gave rise to fewer larvae.

These differences might be an indication of resistance on the part of Doon Star and Epicure plants. It seemed to me, however, that too little was known about the conditions influencing cyst formation to be sure that the differences might not be due to factors affecting plant growth; in fact, they might be a secondary consequence of differences in varietal response to environmental conditions, such as differences in photoperiodic reactions. It was suggested that "these should be investigated before the hypothesis of 'resistance' ... is accepted". The present communication reports the results of an attempt to demonstrate the effect of day-length on cyst formation.

MATERIALS AND METHODS

Plants were grown in a greenhouse, 9ft. × 6ft., one half of which could be 'blacked-out' by means of externally placed shutters. Internally, the two halves could be separated by a partition, part of which con-

sisted of a heavy black curtain which could be drawn into appropriate light traps at the sides. One half of the greenhouse could therefore be 'blacked-out' completely in a matter of minutes. During the day the internal partition was dismantled and the curtain drawn back, and an exhaust fan in the roof of each half helped to circulate the air and also to reduce the interior temperature. When one half was 'blacked-out', the two roof fans were switched off, and an additional exhaust fan sucked air from one half to the other. Two long shallow water-filled troughs, each of which ran the length of the house beneath a staging, helped to keep the atmosphere humid; but the gap between the water surface and the top of the tanks also helped in maintaining a proper circulation of air between the two halves.

This simple set-up was most effective and both the temperature and the humidity of the two halves were uniform except that occasionally an unusually hot afternoon sun would send the temperature at one end above that of the other by about 2° C, when the black-out was not operating.

The experiment here described was carried out in 1952. Tubers of the three varieties Doon Star, Majestic, and Redskin were grown in 9 in. pots of sterile sand. Two ounces of a balanced fertilizer were added to each, in two doses, one before planting and the other when the plants were well established. In all, ten plants of each variety were grown, and five of each variety were placed in each half of the greenhouse. Until the end of June, that is for approximately two months from planting, all the plants were maintained on the same regime exactly, with no reduction of the length of day for any of them. The plants were then all fully grown and examination the previous season of plants at a similar stage, showed that their 9 in. pots were comfortably filled by an extensive root systeem. On June 30, half of the greenhouse was blacked out, as described, from 9 p.m. until 7 a.m. the following morning. The 'black-out' period was lengthened by beginning it an hour earlier each day until, on July 3, it operated from 7 p.m.-7 a.m. It was retained at this length until July 21 and then changed to 5 p.m.-7 a.m. for the remainder of the season.

The plants were inoculated with lots of 400 cysts obtained from the roots of potato plants the previous season. All had failed to pass a sieve of approximately 0.5 mm aperture. They were soaked in water for eight days, mixed with dry sand, and placed in stainless steel gauze baskets (ELLENBY, 1955) of the same mesh as the sieve. Inoculations were made on July 2, that is, soon after the beginning of the establish-

ment of the black-out regime for half of the plants. A hole was made in the sand about an inch from the edge of each pot and an inoculation basket buried almost completely in it. They were removed 19 days later and the inoculation cysts recovered from them. By examination of a random sample of about 25 from each lot of 400 cysts, the number of emerged larvae was estimated for each pot.

When all the plants had died down, the new cysts were recovered from each pot; first from the roots and then from the sand by flotation. Cysts were measured by means of an eyepiece micrometer, and the egg content of a number of individual cysts determined for a sample of the cysts from each pot.

RESULTS

Larval emergence

Emergence of larvae from the lots of 400 inoculation cysts varied from a total of about 8,000, for a Doon Star plant on short days, to almost 32,000 for a short day Majestic plant; mean values are shown in Table I. Analysis of variance, carried out with the totals for the

TABLE I

Emergence of larvae from samples of inoculation cysts

Mean larvae emerged per cyst and S.E. *

Treatment	Variety		
	Redskin	Doon Star	Majestic
Short day	32.0 ± 6.6	29.6 ± 6.6	53.9 ± 7.5
Long day	25.2 ± 6.6	37.3 ± 6.6	44.7 ± 7.5

* Based on error mean square.

samples of 25 cysts of each lot actually examined, showed that length of day had no significant effect, the mean square for day length being about 1/8 of the mean square for error. On the other hand, there was a significant effect ($p = 0.01$) due to variety, Majestic, behaving differently from the other two sorts. For Doon Star and Redskin, the values are fairly similar, but for the Majestic plants they are considerably higher under both treatments. There was no significant interaction between variety or day length (For the variety Majestic hatching information was only available for four out of the five plants on each treatment).

Yield of cysts

As shown in Table II, differences in the yield of cysts were very

striking. Six times and ten times more cysts respectively were found on the Redskin and Doon Star plants on long days than on those on short days; but for Majestic, the mean values for long and short days are almost identical.

TABLE II

Mean number of cysts on three varieties of plant grown under short and long days

Treatment	Variety		
	Redskin	Doon Star	Majestic
Short day	123	123	364
Long day	658	1396	343
S.E. ± 182			

An analysis of variance showed that variety had no significant effect on cyst yield ($p > 0.05$); but length of day had a highly significant effect ($p < 0.001$). Because of the differences in behaviour between Majestic and the other two varieties, it is not surprising that the analysis shows a significant variety-day length interaction ($p < 0.01$).

This contrast in behaviour is strikingly shown if yield of cysts is considered in relation to the number of larvae which emerged in the pots. For Redskin plants on long days, one cyst was formed for every 15 larvae available; for the short day plants, one for every 104. The ratios are similar for the Doon Star plants, one cyst on the long day plants for every 13 larvae, and one for every 96 larvae on the short day plants. For the varieties, therefore, for equal numbers of larvae, seven times more cysts were formed on plants grown under long day treatment than on those grown on short days. With the variety Majestic, however, the case is very different, for, again, there is little difference between the results for the two treatments, one cyst being formed for every 50 larvae available in both cases. It must be stressed, however, that the information concerns the number of larvae which emerged from the inoculation cysts; the proportion of these which actually entered the host plants is unknown.

Apart from differences in actual number of cysts formed on the plants on different treatments, the relationship between cyst number and numbers of available larvae also differed in another important respect. In Fig. 1, values for estimated total emergence have been plotted against cyst yield for the varieties Redskin and Doon Star. On long day (open circles) cyst yield increases with total emergence;

under short days, however, (closed circles) the total number of cysts shows scarcely any trend with increasing emergence. Regression coefficients are 1.077, for the ten long day values, and 0.007 for short days; the former coefficient is highly significant ($p < 0.01$), and the

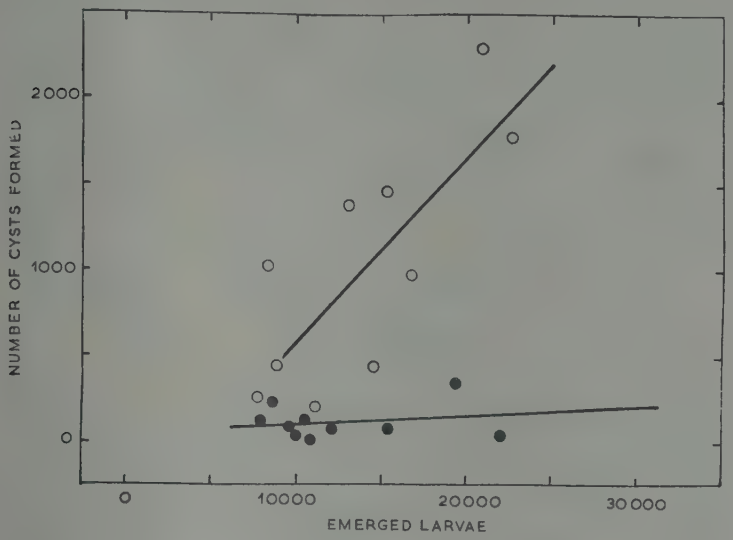

Fig. 1. Larval emergence and cyst yield for varieties Doon Star and Redskin. ○ long day, ● short day.

latter far from significance ($p = 0.4$). Under short day conditions, there was no tendency for cyst numbers to increase with increasing numbers of available larvae.

The results for the Majestic plants have been presented separately in Fig. 2. These apparently show no effect of day length, for the values for both short and long day might easily form a single series.

Cyst volume

The dimensions of the cysts were measured with an eyepiece micrometer, the accuracy of measurement being about ± 3 %. The transverse diameter, D, and the length to the base of the neck, L, were measured and D^2L used as an estimate of relative cyst volume; in practice, the two dimensions coincided for about a third of the cysts. Values of D^2L in micrometer units were used in the calculations to save the labour of the conversions, 10 micrometer units being equivalent to 0.33 mm. In the text, values are given in italics when in

micrometer units; thus, a spherical cyst of 15 micrometer units diameter would have a volume proportional to *3375*, *(log = 3.5283)* in micrometer units, which is equal to 0.065 mm.^{3}.

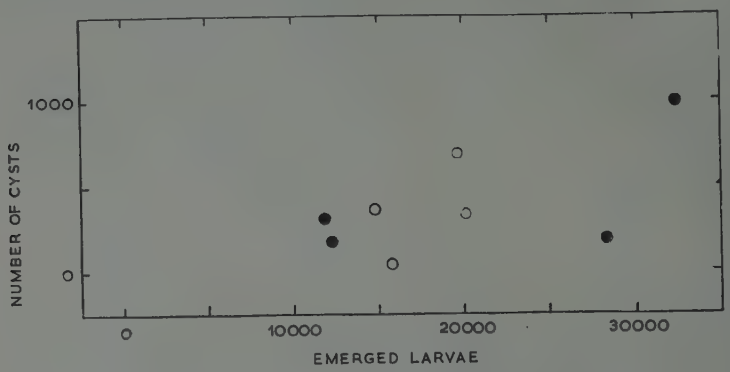

Fig. 2. Larval emergence and cyst yield for variety Majestic.
○ long day, ● short day.

One short-day, Doon Star plant, yielded only 11 cysts. Accordingly, the analyses were based on samples of 11 cysts from each plant.

Because of the occasional large cyst, the analysis of variance was carried out with a logarithmic transformation of values of D^2L. Moreover, the variance for cysts "within" plants was significantly less than that for cysts "between" plants ($p = 0.025$) showing that cysts on the same plant were more similar than cysts on different plants on the same variety and treatment. The analysis was therefore carried out using the sums of the individual values of log D^2L for each lot of eleven cysts.

As shown in Table III, Redskin and Doon Star cysts tended to be smaller on short than on long days; for the latter variety, with S.E. ± 0.07, the difference in mean log D^2L just reaches the level of signi-

TABLE III

Cyst size on three varieties of plant grown under short and long days. Mean values of log D^2L where D = diameter, and L = length of cyst in micrometer units

Treatment	Variety		
	Redskin	Doon Star	Majestic
Short day	*3.163*	*3.152*	*3.190*
Long day	*3.211*	*3.301*	*3.219*
S.E. ± 0.051			

ficance ($p = 0.05$). However, the average differences due to variety or length of day are not significant although, in the latter case, at $F = 3.24$, it approaches the 5 % level of significance.

Cyst volume and egg number

In Fig. 3, values for mean egg number are plotted against mean cyst volume on a double logarithmic grid: closed circles represent values for the 15 plants, five of each variety, on short days, and open

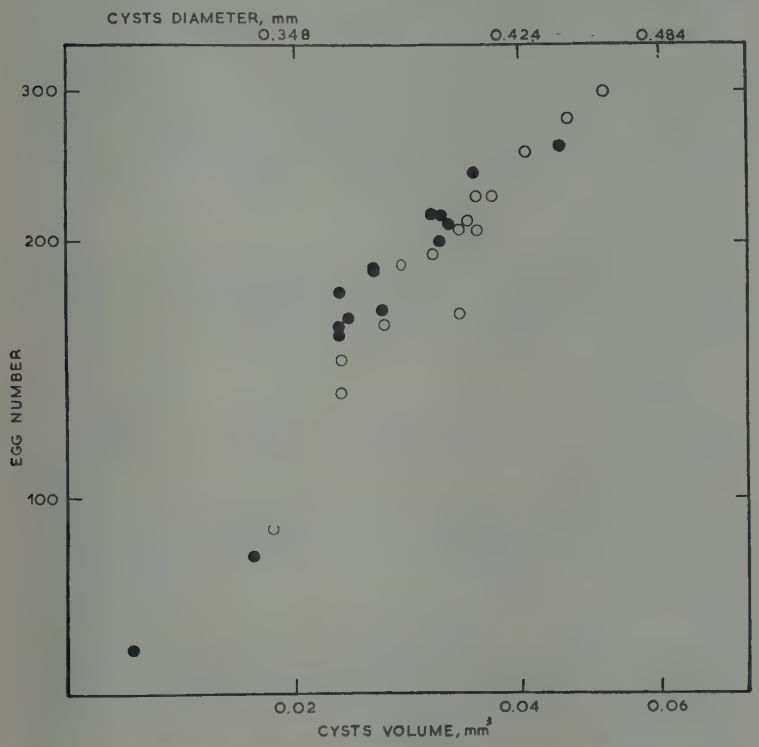

Fig. 3. Egg number and cyst volume on a double logarithmic grid. O values for cysts from plants of three potato varieties on long days, and ● for cysts from plants of these varieties on short days. Diameters shown are for perfect spheres of the same estimated cyst volumes.

circles those on long days. The diagram suggests that the concentration of eggs in the cysts from the short day plants is greater than in the cysts from the long day plants. The size ranges overlap almost completely, and the differences in relative concentration is most clearly shown in the middle of the size range where there are twelve out of

the fifteen short day values and eleven of the long day: the former are almost all above the latter. This suggestion is borne out by the analysis of covariance.

As in the case of cyst volume, it was necessary to carry out both variance and covariance analyses using the totals for each lot of eleven cysts: even after adjustment for cyst size it was found that, in egg concentration also, cysts from the same plant were more alike than cysts from different plants of the same variety and treatment. The separate regression coefficients calculated for each set of five plants, e.g. the five Redskin plants on short days, were all found to be parallel, the mean square for individual slopes being less than that for error: this coefficient, 1.27 ± 0.079, was used in the covariance analysis.

The results of the analysis showed, averaging over the three varieties, that the egg concentration on short days was significantly higher

TABLE IV

Analysis of covariance. Egg content of cysts of similar size. Comparison of adjusted mean values for log egg number at overall mean value of log D^2L, 3.206. Regression coefficient, 1.274.

Treatment	Variety		
	Redskin	Doon Star	Majestic
Short day	2.310	2.283	2.235
Long day	2.225	2.228	2.231
S.E. $\pm$ 0.020			

than on long days ($p < 0.01$). Comparison of the adjusted mean values of Table IV, shows that the differences are largest in the case of Redskin, and substantial for Doon Star; again for Majestic the values are identical. For Redskin the difference is highly significant ($p < 0.01$), and barely below significance for Doon Star ($t = 1.96$). The differences between the average egg concentrations for the three varieties were not significant ($p = 0.2$).

DISCUSSION

The results show that the conditions under which the host plant is grown may influence not only the number and size of the cysts borne upon it, but also their egg concentration and, presumably therefore, their hatching response. Moreover, potato varieties may behave differently: under short day conditions Majestic bore more cysts than the varieties Redskin and Doon Star, but fewer than these varieties under long days. Evidently the term "resistance", if used at all in

these circumstances, must be clearly related to the conditions of growth.

Although the number of larvae which hatched from the inoculation cysts is known, the numbers which actually penetrated the host roots is not. Nor is information available on the relative extent of the root systems and of the distribution of the larvae within them. Since the concentration of parasites in the roots may influence the number of females (ELLENBY, 1954) important information is lacking. There are two facts, however, which suggest that the differences reported are related to the reaction of the host plants to the length of day: both of these are concerned with the behaviour of the variety Majestic.

1. If the results for Redskin and Doon Star were considered on their own, it could be suggested that, under the more favourable growing conditions of long days, the plants were able to develop a more extensive root system and, for that reason, have borne more cysts upon their roots.

But if the variety Majestic is included, such an explanation is untenable, for the plants of this variety bore approximately equal numbers of cysts under both long and short days.

2. Of the three varieties, Majestic plants bore the most cysts under short days, 364 compared with 123 for both the others. It could be suggested that Majestic plants may have had the most extensive root systems when the short day regime was instituted; and this could be supported by the larger number of larvae which this variety stimulated to emerge from the inoculation cysts. But again this explanation is inadequate, for approximately the same number of larvae which gave rise to the largest number of cysts under short days, gave the smallest number on long days, 343, compared with 658 and 1396 for Redskin and Doon Star respectively.

Extent of root can hardly explain these two points. In any case, the root systems were very extensive at the time of inoculation and even the largest number of cysts found in this experiment, 2,308, must be considered a small burden for a fully grown plant. It seems to me, therefore, that an explanation is to be sought in qualitative differences due to the different reactions of the varieties to the change in the photoperiod. Long days have already been shown to influence the reproductive rate of the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood (TARJAN & HOPPER, 1955; GILLARD & VAN DEN BRANDE, 1956).

ACKNOWLEDGMENTS

Thanks are due to Dr. P. S. Hudson, Dr. J. G. Hawkes, and

Dr. K. S. Dodds, for their help with the preliminary stages of this work, and to Dr. D. A. Evans for help and advice on the treatment of the data. A grant from the Agricultural Research Council is gratefully acknowledged.

ZUSAMMENFASSUNG

1. Pflanzen von 3 Kartoffelsorten, Redskin, Doon Star und Majestic wurden auf sterilem Sand in Töpfen gezogen. Das Glashaus war so angelegt, dass die Tageslänge in der einen Hälfte künstlich verkürzt werden konnte, ohne die anderen Bedingungen zu verändern.
2. Alle Pflanzen wurden, bis sie ihre volle Grösse erreicht hatten, bei normalem Tageslicht gehalten. Dann wurde für 50% der Pflanzen jeder Sorte die Tageslänge auf 10 Stunden verkürzt.
3. Jedem Topf wurde ein kleines rostfreies Stahlkörbchen mit 400 einjährigen Cysten zugefügt, nachdem die Pflanzen schon der veränderten Tageslänge unterworfen waren. Die Körbchen wurden nach 19 Tagen wieder entfernt.
4. Die Tageslänge hatte keinen Einfluss auf das Schlüpfen der Larven aus den Inoculationscysten. Hingegen war der Einfluss der Sorte der Kartoffel ganz eindeutig.
5. Die Zahl der Cysten an Redskin und Doon star war kleiner bei reduziertem Tageslicht. Cysten an kurztägigen Pflanzen neigten dazu, kleiner zu sein, enthielten aber proportional mehr Eier.
6. Während die Zahl der Cysten unter Langtag-Bedingungen mit der Anzahl der ausgeschlüpften Larven wuchs, blieb sie unter Kurztag-Verhältnissen beinahe konstant. Die Sorte Majestic verhielt sich ziemlich gleichartig bei beiden Tageslängen.
7. Cysten von einer Pflanze waren einander ähnlicher in bezug auf Grösse und Eidichte als Cysten von verschiedenen Pflanzen, selbst wenn diese von der gleichen Sorte stammten und unter anscheinend gleichen Bedingungen gezogen worden waren.
8. Die Versuche demonstrieren, wie eng die Beziehungen zwischen Parasit und Wirt sind. Die unterschiedlichen Resultate für Majestic und die beiden anderen Sorten lassen sich vielleicht so erklären, dass man hier mit den metabolischen Folgen der verschiedenen photoperiodischen Reaktionen der 3 Sorten zu tun hat.

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Received for publication: March 26, 1957

Nematologica III (1958): 91-96. E. J. Brill, Leiden

DITYLENCHUS MYCELIOPHAGUS N. SP. (NEMATODA: TYLENCHIDAE)

BY

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The destruction of mushroom crops by nematodes was first described by NEWSTEAD (1906) who showed that the causal agent was a species of *Tylenchus*. In 1949 LAMBERT, STEINER & DRECHSLER observed the feeding by *Ditylenchus* sp. on mushroom hyphae; BOVIEN (1951) identified the species as close to, if not identical with, *D. destructor* Thorne, 1945. SEINHORST & BELS (1951), after a series of experiments comparing the effect on mushroom mycelium of the nematode from mushroom spawn with that of *D. destructor* from potato, concluded that the mushroom nematode was *D. destructor*. CAIRNS (1955) showed that sterilised specimens of *Ditylenchus* sp. caused damage to, and ultimately, destruction of, mushroom mycelium. PAESLER (1957) referred to the mushroom spawn nematode as *Ditylenchus* sp. and CAIRNS (1953), in a thesis, made a similar reference.

For the last few years the author has been referring to the *Ditylenchus* sp. attacking mushroom mycelium as *D. destructor* but recently, close examination has revealed a number of characters by which it is plainly different from *D. destructor*. TARJAN & HOPPER (1954) referred to a *D. mycophagus* from mushroom compost. Correspondence showed that this was a *nomen nudum*, but it was the name under which CAIRNS was intending to publish a description of the *Ditylenchus* attacking mushroom spawn. Unfortunately circumstances have prevented CAIRNS from publishing the description so, by mutual agreement, the present author has undertaken to do so. *D. mycophagus* is ineligible so the name *Ditylenchus myceliophagus* is proposed for the mushroom spawn nematode.

All measurements, in the description which follows, were made on specimens fixed in 5 percent formalin or TAF and mounted in glycerine.

Ditylenchus myceliophagus n. sp.

Holotype ♀ L = 0.87 mm., a = 30.6; b = 7.1; c = 14,2, V = $\frac{61}{80.5}$ $\frac{4.1}{}$

Allotype ♂ L = 0.76 mm., a = 40.5; b = 5.8; c = 12.4; T = 74

Cuticle finely but obscurely annulated (under oil immersion), striae about 1.4 μ apart, and half that distance apart on the subcuticle; however, under low magnifications striations are difficult to see. Lateral fields with six incisures. Cairns (1953) reports six and adds that each area between the incisures is often marked by a longitudinal line making eleven incisures in all: incisures never very easy to observe. Head not annulated and not offset from the body, rather flattened and cap-like, very similar to that of *D. destructor*. Spear about 14 μ long and hardly distinguishable into the usual three sections as it gradually widens from its tip to end in posteriorly directed knobs; junction of the dorsal oesophageal gland lumen very close to spear base. Median bulb of oesophagus fusiform, terminal bulb broadly spathulate and overlapping one side of intestine for a short distance. Female gonad figured in Fig. 1e; except for a short length near its anterior end it consists of a single row of cells. There is an oval spermatheca. Vulva a transverse slit and post-vulval sac extending about halfway to vulva. Eggs about 50 by 22 μ and nearly two body diameters long. Tails of both sexes taper gradually to a broadly pointed, finger-like end but male tail is sometimes much more like that depicted for *D. destructor* (Fig. 1a).

Differentiation. In the accompanying figures the main differences between *D. myceliophagus* and *D. destructor* are depicted. The easiest to see are the relative shapes and sizes of the tails of both sexes, the spicule shape and the relative length of the post-vulval sac. These taken with the differences in the number of incisures are the main grounds on which the two species are separated.

Comparisons have been made between specimens of the mushroom spawn nematode from this country, the Netherlands and U.S.A. and, on morphological and pathogenic grounds, they are all obviously the same species. The American ones were a culture of those that Cairns had worked with and the Dutch ones came from compost obtained by Dr. Oostenbrink. In the accompanying tables some of the dimensions and body ratios pertaining to these populations are set out and compared with similar data for *D. destructor*. These tables emphasise the

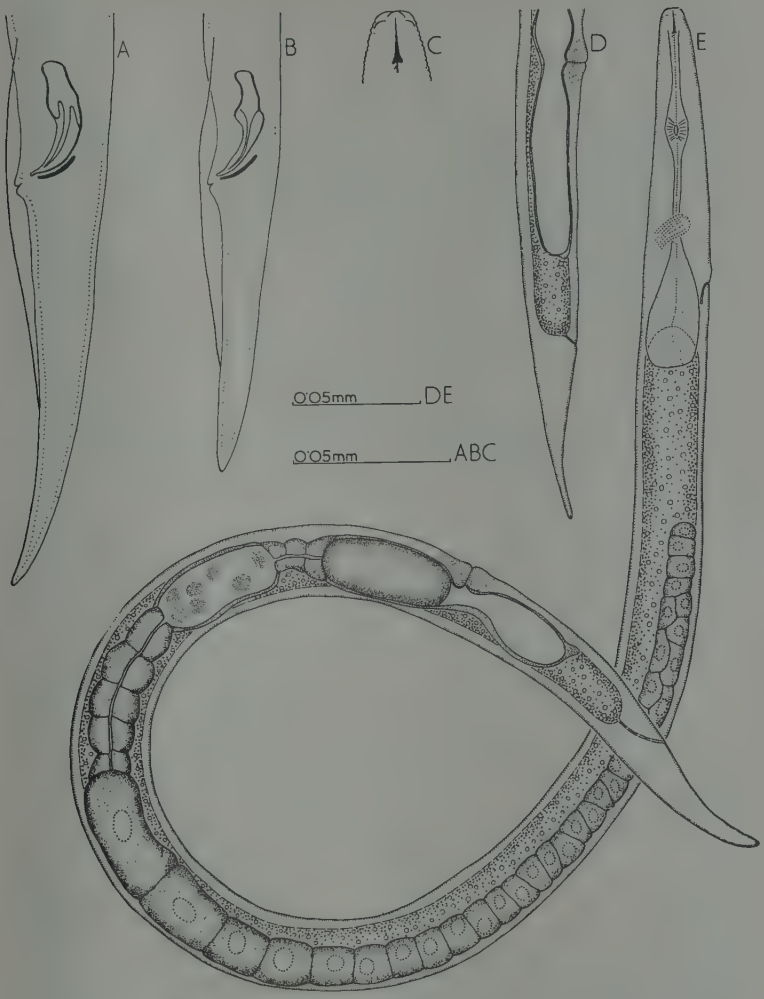

Fig. 1. *Ditylenchus myceliophagus* n.sp., E female; B male tail; C head and spear. *D. destructor*, A male tail; D female hind end.

TABLE I

Dimensions of five populations of Ditylenchus myceliophagus showing means and ranges

Origin of population	sex	n	l	a	b	c	V	
Leicestershire	♀	25	1.01 (0.79-1.38)	31.2 (27.8-38.8)	8.4 (6.0-11.7)	15.1 (11.6-19.7)	58 (47-75)	82.8 (80.4-84.9)	4.5 (3.3-5.7)
Kent	♀	25	0.81 (0.73-0.93)	30.9 (23.8-37.4)	7.1 (5.6-8.6)	14.6 (11.1-17.6)	44 (35-54)	84.0 (78-86)	5.7 (4.6-6.7)
Netherlands	♀	25	0.82 (0.76-0.95)	28.9 (23.6-35.5)	6.5 (5.5-7.6)	12.9 (10.5-16.5)	59 (46-66)	82.0 (79-86)	4.2 (2.9-5.5)
U.S.A.	♀	25	(0.81-1.00) 0.91	(22.6-36.1) 29.4	(5.4-8.6) 7.4	(11.1-20.5) 14.8	63 (55-72)	83.5 (79-90)	5.0 (3.4-5.9)
U.S.A. (CAIRNS 1953) [1]	♀	?	0.75 (0.6-0.9)	33.8 (23.1-44.4)	8.0 (6.4-9.5)	14.5 (12.2-16.8)		80.4 (73.9-86.9)	
								T	
Leicestershire	♂	25	0.76 (0.63-0.88)	36.2 (27.0-44.4)	6.5 (4.6-8.0)	13.2 (10.7-15.3)		60 (53-71)	
Kent	♂	25	0.65 (0.58-0.75)	30.8 (21.4-41.0)	5.5 (4.5-6.9)	11.8 (9.5-14.5)		47 (36-58)	
Netherlands	♂	25	0.69 (0.63-0.77)	34.7 (27.2-41.5)	5.8 (5.0-6.6)	11.0 (9.6-13.2)		65 (53-73)	
U.S.A.	♂	25	0.74 (0.66-0.82)	31.5 (26.2-40.0)	6.0 (5.1-7.1)	13.4 (11.2-15.1)		59 (51-70)	
U.S.A. (CAIRNS 1953) [1]	♂	?	0.7 (0.6-0.8)	30.0 (27.7-32.2)	7.8 (6.8-8.7)	13.3 (11.6-15.0)		73.5 (69-78)	

1 Means calculated from ranges.

great similarity between *D. myceliophagus* and *D. destructor,* the only difference of any note being shown by the data for the post-vulval sac in Table II. This confirms what has already been pointed out in the figures.

TABLE II

Summarized data from populations of D. myceliophagus compared with similar data for D. destructor

Nematode	sex	n	l	a	b	c		V	
D. myceliophagus	♀	100	0.889	30.1	7.3	14.4	56	82.5	4.8
D. destructor[1]	♀	237	1.070	31.6	7.2	16.5	54	80.3	8
								T	
D. myceliophagus	♂	100	0.709	33.3	5.9	12.3		57.8	
D. destructor[1]	♂	231	0.960	35.1	6.7	14.0		65.1	

[1] Figures from GOODEY 1952.

TYPE HOST. Mycelium of *Agaricus hortensis* Cooke, the cultivated mushroom.

TYPE HABITAT. Mushroom compost in Britain, Netherlands and U.S.A.

TYPE SLIDES. Holotype ♀ slide no. 53/11/1, Allotype ♂ slide no. 53/11/2 in the collection of the Nematology Department, Rothamsted Experimental Station.

BIONOMICS. One of the reasons why the author, for so long, considered the mushroom spawn nematode to be *D. destructor* was its apparent inability to withstand drying and to revive on remoistening. Late in 1955 Mr. B. D. MORETON informed the author, in a letter, that he had been able to revive nematodes which had been dry for over two years in compost. Experiment showed that, provided the nematodes dried out slowly, over a period of days or weeks, they would revive on moistening. When, however, the nematodes were dried rapidly in a few hours they would not revive on moistening. CAIRNS (1953) has shown that *D. myceliophagus* forms dried masses "curds" (similar to eelworm wool), and that on moistening, after different periods in anabiosis, the eggs became non-viable shortly before 50 days, first and second stage larvae were non-viable at 100 days whilst older larvae and adults remained viable for a considerably longer time. *D. myceliophagus* thus occupies a position midway between *D. dipsaci,*

the pre-adult larvae of which will revive after rapid drying, and *D. destructor* which will not survive drying at all.

ACKNOWLEDGEMENTS

Thanks are offered to Dr. E. J. Cairns for supplying the culture of *D. mycceliophagus* from U.S.A., to Mr. B. D. Moreton for the sample of diseased mushroom compost from Kent, to Dr. M. Oostenbrink for the sample from the Netherlands and to Mr. F. C. Wood for the sample from Leicestershire. I am also grateful to Mr. D. J. Hooper for mounting nematodes and for making the measurements on which the tables are based.

ZUSAMMENFASSUNG

Ditylenchus myceliophagus n.sp., der Pilzmyzel vernichtet, wird beschrieben und abgebildet. Er unterscheidet sich von seinem nächsten Verwandte, *D. destructor*, durch den breiteren fingerähnlichen Schwanz in beiden Geschlechtern, durch die Form der Spicula und den kurzen postvulvaren Sack.

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Received for publication: November 25, 1957

Nematologica III (1958) : 97-107. E. J. Brill, Leiden

HOPLOLAIMUS GRACILIDENS, RADOPHOLUS INAEQUALIS, AND *RADOPHOLUS NEOSIMILIS,* THREE NEW TYLENCHS NATIVE TO VICTORIA, AUSTRALIA

BY

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For some years the Commonwealth Research Station, Merbein, has maintained a plot of land in uncleared mallee forest near Lake Mournpool, Hattah, Victoria, where observations on the growth of native vegetation are made periodically. The region is semi-arid (rainfall about 25 cm. per year), with very sandy soils. The dominant plant species are mallee trees—species of *Eucalyptus* L'Hérit.—attaining a height of some three to five metres, and porcupine grass (*Triodia irritans* R. Br). Much of the ground surface is bare for many months of the year.

Soil samples taken in September 1956, contained three new species of tylenchs which are now described. Three more series of soil samples have been taken subsequently in this vicinity to obtain more specimens and gain information about their distribution. Each series of samples included soil from the original sites and from several new sites.

Radopholus inaequalis n. sp. requires special mention. No males were found in the original samples. Certain similarities in general morphology were apparent between this species and the much disputed *Rotylenchus obtusus* (Bastian) Filipjev 1936, which has lately been confirmed as a species of *Rotylenchus* by Goodey & Franklin (1956). Detailed examination and the recovery of a few males confirm that the species from Hattah, despite these similarities, is indeed a *Radopholus* with rather distinctive features.

Three males and several gravid females of *Radopholus inaequalis* were found in a sample taken in the fourth series from the original site in July 1957. Females with eggs had previously been seen only in the original samples. The July samples also contained the two males of *Radopholus neosimilis* which are described (one other male, from the original sample, was not available for description). Samples

taken in February showed relatively many larvae and few adults of *Radopholus,* whereas adults of *Hoplolaimus gracilidens* were common in each series of samples.

These species are the first *Radopholus* recorded in Victoria, though *R. similis* (Cobb) Thorne 1949 has been recorded as occurring in Queensland by Colbran (1955). From the isolated and untouched nature of the site at Hattah it is clear that the species now described are native to that area.

Hoplolaimus gracilidens new species [1]) (Fig. 1).

Measurements: 20 ♀♀, L = .950 to 1.200 mm; a = 27-39; b = 7.0-9.8; c = 33-61; V = 53-57 %.

10 ♂♂, L = .900 to 1.100 mm; a = 33-40; b = 7.0-8.6; c = 30-35.

Description: Body almost cylindrical, but tapering from latitude of oesophageal lobes to head; generally assuming a spiral shape when relaxed by heating. Cuticle coarsely annulated. Tail short, but very variable in length, bluntly rounded. Cuticular annulations distinct around terminus.

The lateral fields are about one quarter body width divided into three equal parts by four incisures. The incisures vary from smooth to crenulate and may be irregular on the tail. In the oesophageal region the body annulation continues right across the lateral field, but over most of the body the annulation is seldom continued into the lateral field, and never beyond the second of the four lateral lines.

In the female the phasmids appear in the lateral fields as small dots, some two anal diameters anterior to the anus. Left and right phasmids are at the same latitude. The male phasmids are somewhat anterior to the bursa.

Head set off from body by a distinct constriction which is more prominent in males than in females. Framework of head strong, yellowish. The head bears four annules in both sexes, but shows distinct sex differences. The female head is rounded, the male more or less cylindrical. In face view twelve small papillae are visible at the margins of the labial disc. Amphids are inconspicuous. The head is deeply divided into six lobes; in the male the two lateral lobes are much

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1) Recently Loof & Oostenbrink (*Nematologica* **3**, 1958, p. 34-43) have shown that *Hoplolaimus uniformis* Thorne is synonymous with *Tylenchus robustus* De Man and therefore is type species of *Rotylenchus.* As *Hoplolaimus gracilidens* n.sp. is congeneric with this species, (cf. also Andrássy, ibid. p. 44-56) the proper name for the former species is *Rotylenchus gracilidens.* (Note added in proof).

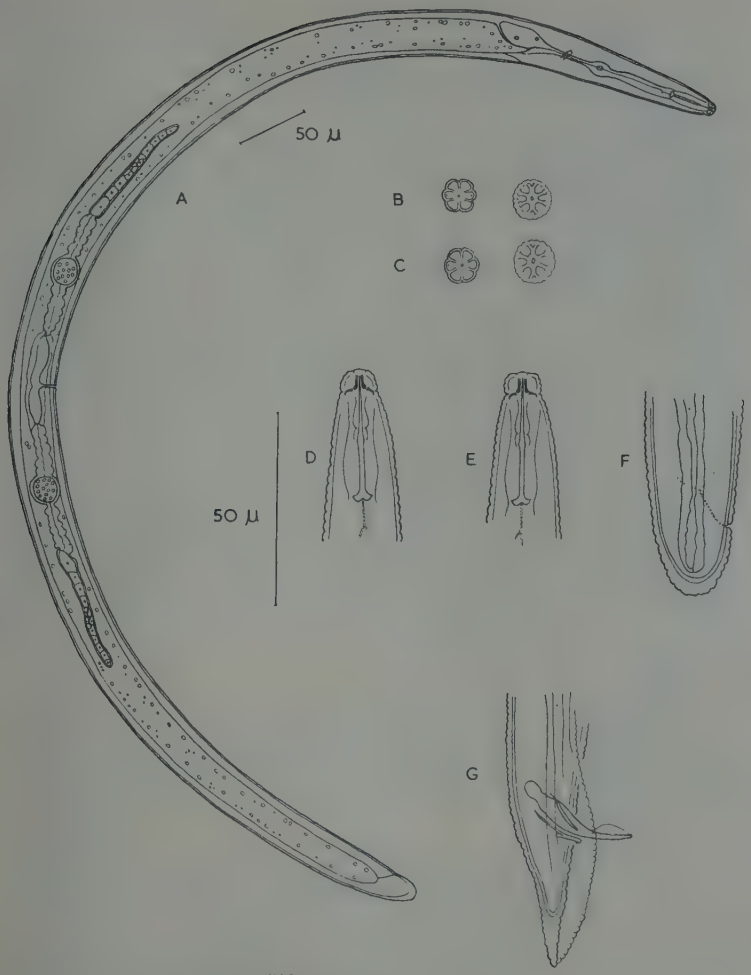

Figure 1: *Hoplolaimus gracilidens* n. sp.

A: female
B: face view and basal plate, male
C: face view and basal plate, female
D: anterior end, female
E: anterior end, male
F: female tail
G: male tail

reduced in size, but in the female the lobes are sub-equal. In both sexes the basal plate is hexa-radiate with dorsal and ventral ribs dividing into two arms. The lowest annule of the head is divided into approximately 21 lobes by striations longitudinal to the body.

Spear quite strong, though slender for this genus, some 34-38 microns long. The spear knobs are swept back to distinct points. The dorsal oesophageal gland opens some six microns posterior to the spear knobs. The corpus of the oesophagus is slender, with median bulb and valve of moderate size. Isthmus rather long, surrounded by nerve ring. Posterior lobed portion of oesophagus short, structure indistinct, nuclei of glands often obscure, two nuclei sometimes visible. The excretory pore opens opposite the oesophageal lobes. The junction of oesophagus and intestine is in the same latitude, but is often obscured. No hemizonid observed.

The vulva is a transverse slit about half as wide as the body, equatorially placed. Ovaries paired, outstretched, sub-equal, each usually some 20-30 % of total body length. In each ovary a well defined uterus leads to a long cellular oviduct including a distinct round spermatheca, usually well filled with spermatozoa. Eggs not seen. Oocytes in a single line except for region of multiplication.

Testis single, outstretched, occupying some 38-50 % of total body length. Spicules large, strong, with well developed membranous process at their points. Gubernaculum almost half as long as spicules, cephalate, bearing titillae. Telamon well developed. Bursa large, enveloping tail tip.

Diagnosis:

Hoplolaimus, with the usual characteristics of the genus, distinguished from species already described by the forward placing of the small phasmids, by its small size, and by the relatively short and slender spear.

Type locality:

Soil about the roots of *Olearia lepidophylla* Benth. in virgin mallee scrub near Lake Mournpool, Victoria.

This species is common in small numbers about the roots of a number of native plants on sandy soils in uncleared mallee areas around Hattah.

Radopholus inaequalis new species (Fig. 2).

Measurements: 20 ♀♀ L = .510-.750 mm; a = 22-30; b = 6.0-8.9; c = 11.5-16; V = 54-62 %

3 ♂♂ L = .520, .530, .590 mm; a = 30, 31, 33; b = 6.2, 6.4, 6.8; c = 16.8, 16.5, 15.5.

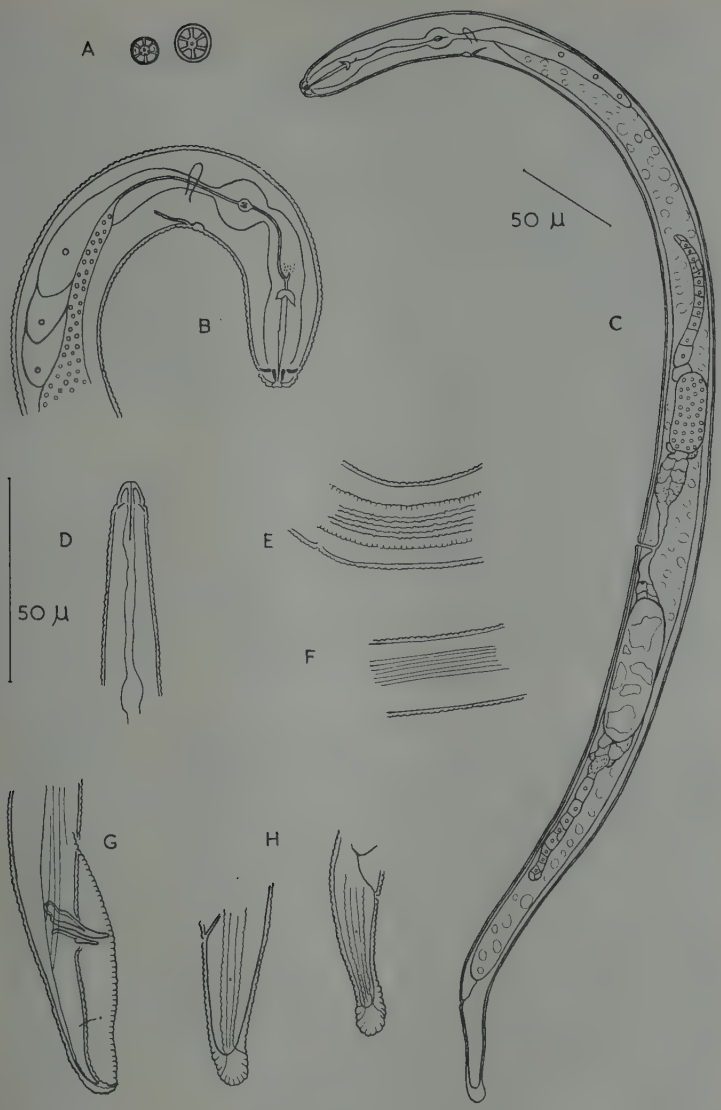

Figure 2: *Radopholus inaequalis* n. sp.

A: face view and basal plate, female
B: anterior end, female
C: female
D: head end, male
E: lateral lines, female
F: lateral lines, male
G: male tail
H: female tails

DESCRIPTION: Marked sexual dimorphism.

Female: Body more or less cylindrical, tapering at the tail, the anterior portion rounded abruptly to the rather small head. Cuticle strongly annulated. Tail two or three times as long as anal body diameter. Body annulations are distinctly continued around terminus which is bluntly rounded or clavate.

The lateral fields are one third to one half body width, marked by numerous distinct incisures. The number of lines increases from the oesophageal region by inclusion of extra lines in the middle of the body, decreasing again to four in the anal region. The inner lines vary from very irregular to almost smooth and may terminate in a series of short oblique lines. They are often broken, sometimes appearing as oblique striae in the vulvar region. The outer incisures are usually crenate, often crossed by body annulations, and appear continuous with the striations around the tail.

The phasmids appear as small dots in the lateral fields about one third tail length posterior to the anus.

Head small, flat, sharply rounded in front, distinctly set off from body. The head bears three annules (2 striations) which are often irregular and difficult to count. It has a strong framework. There are six papillae situated at the top of the supporting ribs, visible in lateral or face view. Amphids obscure.

The spear is very robust, 22-24 microns long, with large basal knobs. The dorsal knob is much larger than the ventro-lateral knobs, and all are sharply pointed anteriorly. The opening of the dorsal oesophageal gland is some 3-4 microns behind the base of the spear knobs. The median oesophageal bulb is large and round, with large valve. The nerve ring encircles the isthmus. The posterior lobed portion of the oesophagus is very long, overlapping the intestine dorso-laterally, and its exact structure is usually obscured. It consists of three distinct overlapping lobes, not one long lobe as described for *Radopholus similis* by THORNE (1949). The arrangement appears to be similar to that described by COBB (1915). The excretory pore opens near the anterior end of the oesophageal lobes. The hemizonid is very distinct, about two body annules in width, situated immediately in front of the excretory pore or one or two annules anterior to it.

The intestine usually contains many globules which often obscure details of the reproductive system. In many specimens certain details were obscured by spores of a sporozoan parasite.

The vulva is a transverse slit, one third as wide as the body, with

slightly protruding lips. Ovaries outstretched, anterior and posterior branches subequal, each some 25-30 per cent. of body length. Each ovary has a short uterus, long oviduct, large ovoid spermatheca, and oocytes mostly in a single row. Egg 65-70 microns by 20 microns.

Male: Only three males of this species have been recovered, with several hundred females and larvae, from several lots of soil samples.

Body with a pronounced anterior taper; quite slender at the head end. Cuticular annulations much less pronounced than in females. Head narrow, quite elongate, rounded in front, apparently unstriated. Framework much reduced, cuticularisations at base of head prominent. The first three or four annules behind the head somewhat distended. Spear and oesophagus degenerate; spear knobs minute, oesophageal valve not seen. Hemizonid prominent, marked by a distension of the cuticle. Lateral fields up to one third body width, marked by about 7 smooth faint lines. The dorsal line extends to the tail tip and may be crenate in the region of the bursa. Several of the other lines may terminate obliquely at the latitude of the spicules.

Testis single, outstretched, 45-50 per cent. of total body length. Bursa voluminous, enveloping tail tip. Spicules slender, arcuate. Gubernaculum stout, about half spicule length, with a distinct hook at the proximal end. Phasmids located on bursa about half way from spicules to tail tip.

DIAGNOSIS:

Radopholus showing marked sexual dimorphism. Females distinguished from species already described by the presence of up to 7 lines in the lateral fields, by the relatively stout spear with unequal, pointed knobs, by the small rounded head, and by the striations around the tail tip.

The male is characterised by the elongate unstriated head, multiple lines in the lateral fields, and voluminous bursa.

TYPE LOCALITY:

Soil about the roots of *Codonocarpus cotinifolius* F. v. M. in virgin mallee scrub near Lake Mournpoul, Victoria. *Codonocarpus* has a relatively limited distribution in the mallee around Hattah, but *Radopholus inaequalis* has been recovered from about the roots of all *Codonocarpus* trees examined, from several widely separated sites.

Radopholus neosimilis new species (Fig. 3).

Measurements: 12 ♀♀ L = .510-.675 mm; a = 24-33; b = 5.7-6.7; c = 14-16 V = 56.5-61 %

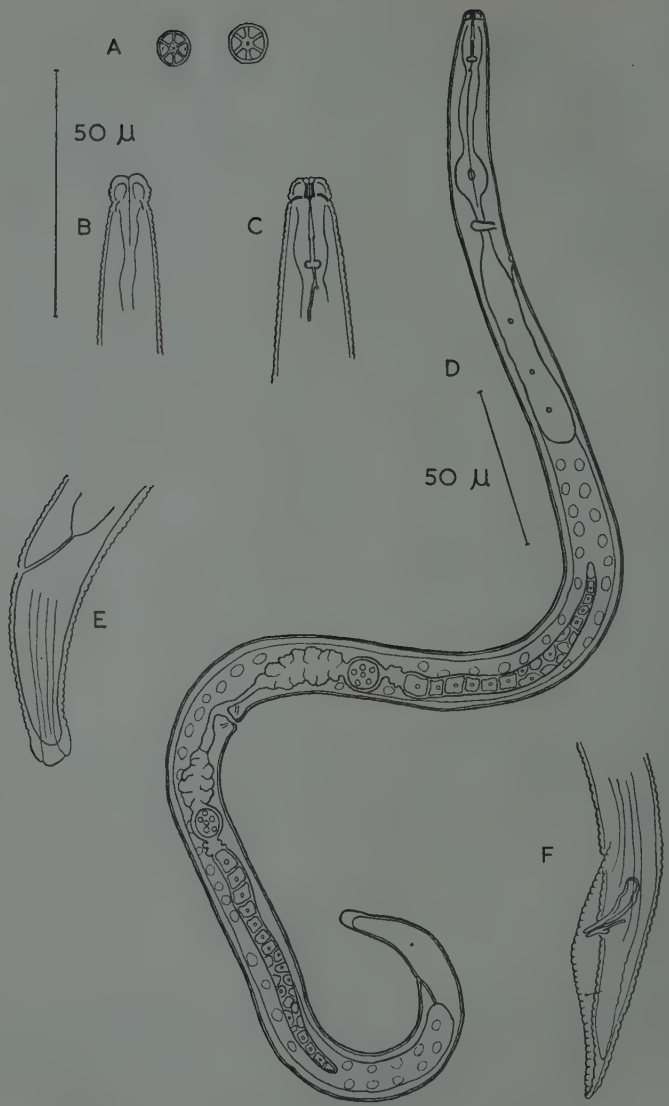

Figure 3: *Radopholus neosimilis* n. sp.

A: face view and basal plate, female
B: male, anterior end
C: female, anterior end
D: female
E: female tail
F: male tail

2 ♂♂ L = .390, .465 mm; a = 30, 31; b = 5.8, 6.1; c = 14.5, 14.

DESCRIPTION: Marked sexual dimorphism.

Female: Body cylindrical, tapering at the tail, anterior portion conical with the head conforming to the general body contour. Cuticle strongly annulated. Tail two or three times as long as anal body diameter, bluntly rounded. Terminus smooth or showing slight striations.

Lateral fields about one third body width, bearing four incisures. The inner incisures are more or less smooth, the outer two are crenate. The outer lines at least, sometimes all four, appear to continue around the terminus.

The phasmids appear as small dots in the lateral fields about half way from the anus to the tail tip.

The head is broad, flat, slightly rounded anteriorly, and set off from the body. It bears four annules (3 striations) which are usually clearly visible. There are six small papillae at the top of the supporting ribs. Amphids obscure.

Spear strong, 16-18 microns, with large rounded basal knobs. The dorsal oesophageal gland opening is some 4 microns behind the base of the spear knobs. The median oesophageal gland is large and subspherical. The large valve is usually anterior to the centre of the bulb. Isthmus long, encircled by nerve ring. Lobed portion of oesophagus very long, exact structure not seen. The excretory pore opens opposite the lobed part of the oesophagus. The hemizonid is large, often situated several body annulus in front of the excretory pore. Junction of the oesophagus and intestine usually difficult to see.

Vulva a transverse slit about one third as wide as body. Ovaries paired, outstretched, approximately equal, and each some 20-30 per cent. of body length. A distinct round spermatheca, usually well filled with sperms, is present in each ovary. The oocytes are in a single row except for a rather long region of multiplication. Eggs not seen.

Male: Body more or less cylindrical, cuticle coarsely annulated. Lateral fields marked by four lines, the outer lines more or less crenate. The dorsal line continues to the tip of the tail, but the others terminate in the region of the spicules. Head elongate, rounded in front distinctly set off from body. It comprises three clear basal annules, and a large unstriated anterior portion. Framework of head reduced, but basal cuticularisations distinct. Spear and oesophagus much reduced, neither spear knobs nor oesophageal valve seen. Hemizonid prominent.

Testis single, outstretched, about 45 per cent. of total body length. Bursa rather narrow, enveloping tail tip. Spicules arcuate, sharply

pointed. Gubernaculum about half spicule length. Phasmids located on bursa about half way from spicules to tail tip.

DIAGNOSIS:

Radopholus resembling *R. similis* rather closely, but somewhat smaller, and readily distinguished from *R. similis* by the shorter, blunter tail of the female, and the lower, broader head and shorter tail of the male.

Female characterised by conical anterior portion of body, broad flat head with 4 annules, round spear knobs, four lines in lateral fields, short, bluntly rounded tail.

Male characterised by rounded head with distinct annules, 4 lines in lateral field, narrow bursa enveloping tail tip, testis about 45 per cent. body length.

TYPE LOCALITY:

Soil about the roots of *Codonocarpus cotinifolius* in virgin mallee scrub near Lake Mournpoul, Victoria. This species has been found in small numbers about the roots of several *Codonocarpus* trees in the Hattah area but does not seem as common as *R. inaequalis.*

TYPE SPECIMENS:

Type specimens of the three species described are designated as follows:

Hoplolaimus gracilidens:

Holotype: Female, slide *Hoplolaimus* 3, collection of Commonwealth Research Station, Merbein.

Allotype: Male, same data as female.

Radopholus inaequalis:

Holotype: Female, slide *Radopholus* 1, collection of Commonwealth Research Station, Merbein.

Allotype: Male, slide *Radopholus* 2, C.R.S., Merbein.

Radopholus neosimilis:

Holotype: Female, slide *Radopholus* 3, C.R.S., Merbein.

Allotype: Male, slide *Radopholus* 4, C.R.S., Merbein.

ACKNOWLEDGMENTS

The author wishes to express his thanks to Dr. J. W. Seinhorst, Dr. J. B. Goodey, Dr. A. L. Taylor, and Professor Gerald Thorne, each of whom took the trouble to examine certain specimens on his behalf, and offered helpful advice.

Mr. L. Smith, of Commonwealth Research Station, Merbein, washed most of the soil samples for the author.

ZUSAMMENFASSUNG

Drei neue Tylenchusarten aus nicht urbar gemachtem Waldboden in Nord-Viktoria (Australien) werden beschrieben. Das Gebiet ist semiarid mit sehr sandigen Böden. Kleine Bäume der Gattung *Eucalyptus* L'Hérit. und Klumpen des Grases *Triodia irritans* R. Br. sind die vorherrschenden Pflanzen.

Hoplolaimus gracilidens n.sp. ähnelt *H. uniformis*, unterscheidet sich aber durch geringere Grösse, schlankeren Stachel und hervortretende kleine Phasmiden beim Weibchen, zwei Analdurchmesser vor dem After. Die Kopfform variiert mit dem Geschlecht. Die Originalstücke wurden aus Boden um Wurzeln von *Olearia lepidophylla* Benth. erhalten, aber die Art war in geringer Zahl um Wurzeln mehrerer einheimischer Pflanzen allgemein verbreitet.

Radopholus inaequalis n.sp. und *R. neosimilis* n.sp. gehören beide dem *R. similis*-Typ an mit deutlichem Sexualdimorphismus. Das Weibchen von *R. inaequalis* weist drei Ringe am Kopf, 5-7 Seitenlinien, kräftigen Stachel mit spitzen ungleichen Knöpfen und einen kurzen, stumpfen Schwanz mit geringelter Spitze auf. Das Männchen hat einen reduzierten Stachel und Oesophagus, einen verlängerten nichtgestreiften Kopf, etwa 6 oder 7 schwache Seitenlinien und eine die Schwanzspitze umfassende Bursa.

Das Weibchen von *R. neosimilis* ähnelt *R. similis* mit 4 Ringeln am Kopf, 4 Seitenlinien und flachen Stachelknöpfen. Es unterscheitet sich durch einen kurzen stumpfen Schwanz mit glatter oder etwas gestreifter Spitze und durch geringere Grösse. Das Männchen hat einen reduzierten Stachel und Oesophagus, breit gerundeten Kopf, etwas Ringelung an der Basis und eine die Schwanzspitze umfassende Bursa. *R. inaequalis* und *R. neosimilis* wurden aus Boden um Wurzeln von *Codonocarpus cotinifolius* F. v. M. von verschiedenen Orten erhalten.

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Received for publication: September 24, 1957

Nematologica III (1958): 108-114. E. J. Brill, Leiden

THE PRODUCTION OF ROOT DIFFUSATE BY POTATOES GROWN IN WATER CULTURE

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As an alternative to growing potatoes in sand or in soil for root diffusate collection, attempts have been made in three experiments to produce it directly by growing them in a small volume of nutrient solution or water, according to the method by D'HERDE, KIPS & VAN DEN BRANDE (1956). The same methods of plant culture and diffusate assay were used throughout.

MATERIALS AND METHODS

Tubers of the variety Arran Banner each $1^1/_2$ oz. in weight were grown in pots of sand. A few days after emergence of the shoots, selected plants were shaken out of the pots, the roots were washed, and each plant was placed over one litre of nutrient solution in a jar of 1,250 ml. capacity. The plants were supported by mounts made from „Halna" plant pots, which are manufactured from a bitumastic and paper mixture (See Fig. 1). They were fastened into the mounts by passing a string through the tuber and the walls of the mount, which then rested in the neck of the jar and supported the plant above the solution. The tubers and young roots were thus enclosed in a humid atmosphere and the shoot grew up through a hole in the "lid". The jars were painted black to exclude the light.

The formula of the nutrient solution is as follows: Calcium nitrate ($Ca(NO_3)_2.4H_2O$) 1.69 g., Sodium dihydrogen phosphate 0.194 g., Potassium sulphate 0.335 g., Magnesium sulphate 0.505 g., Boric acid 0.00545 g., Manganous sulphate 0.00396 g. per litre. These weights gave Ca 287, N_2 200, Na 37, P 50 (PO_4 154), K 150, Mg 50, B 1, and Mn 1, parts per million, and a pH of approximately 5.5 when made up in distilled water. To avoid precipitation of ferric phosphate (HOUGHLAND 1947) iron was supplied in 0.5 % Ferrous sulphate ($FeSO_4.7H_2O$) as a foliar spray twice weekly. The fluid level in the jars was maintained by the daily addition of distilled water. The solutions were not aerated.

Fig. 1. Showing potato plants growing in the jars of nutrient solution after 4 and 32 days. The labels on the jars refer to treatments and not to age.

The diffusates were assayed in hatching tests by the method of FENWICK & WIDDOWSON (in press). Replicated batches of 100 cysts containing approximately 140 eggs and larvae per cyst were used. About 85 % of the total cyst contents were 'hatchable' in undiluted standard diffusate.

In a preliminary trial the plants were grown in a shaded glasshouse and the temperature in the jars occasionally rose from about 14° C to 25 or 30° C during the day. Accordingly, to avoid damaging the plant and losing hatching factor (FENWICK 1957, NEWTON 1923), the plants were transferred to outdoors with the jars immersed in 4 in. of water. Here the rate of growth, and the condition of the roots was satisfactory and a temperature of 10-14° C was maintained, at which diffusate is stable for short periods.

RESULTS

The first experiment was set up to estimate the diffusate output of potatoes grown in water and to compare with it that from potatoes grown alternately in water and in nutrient solution; replication was three fold. At transplanting, three mounted plants were placed over water and twenty four over nutrient solution. The water plants were the controls from which the daily output of diffusate was estimated by changing the water every day. After one day, six of the plants in nutrient solution were transferred to water. Three then received the same treatment as the controls, and three were given alternate days in nutrient solution and water. On the following day, six more plants were transferred to water, three remained in water and three were alternated between water and nutrient solution every two days. Similarly after four and eight days in nutrient, six plants were divided between water which was changed daily, and between water and nutrient solution alternately every four days, and every eight days, respectively. By leaving the plants in water for 2, 4 and 8 days without disturbance the concentration of diffusate was allowed to build up. The same jar of nutrient solution was used for eight days by each plant and then the spent solution was discarded.

In this way, the daily production of diffusate in water without feeding, after 1, 2, 4 and 8 days initial feeding and with continual but interrupted feeding for the same periods could be estimated. Diffusate production was traced by taking 20 ml. samples periodically from the jars of water. The nutrient solution was not assayed for hatching activity.

Production was slow for the first two weeks of the experiment but during the fourth week, diffusate of L.A. 3.4 (mean of three replicates) was produced by the plants alternated between nutrient solution and water every other day compared with diffusate of less than L.A. 3.0 from the other treatments. Production then declined generally, the final nine week values being approximately L.A. 1.4 daily from the controls and between L.A. 1.0 and 2.0 for the rest. There was no evidence of build up in activity in the jars left undisturbed for two, four and eight days, and although the results suggested that the alternated, one-day feeding had increased diffusate output there was no real difference between the root weights of this and the other treatments at the end of the experiment.

In the second test the plants were grown continually in nutrient solution to encourage rapid root growth, replication was two fold and ten plants were used. Eight were transplanted to nutrient solution and two control plants were left to grow on in the pots of sand. The plants were grown in the open with the pots and jars plunged in gravel, and were left for eight days initially to allow the roots to grow down to the solution which was discarded and replaced with fresh at the start of the experiment. The root temperature varied between 12-16° C. The potted potatoes were leached with 1 litre of water daily and 50 ml. of nutrient solution was watered on to the pot after leaching. The pattern of diffusate output from the water-culture plants was the same in all treatments with the highest activities occurring after four weeks of growth as happened in the first experiment. However, production in the jars was not comparable with that from the pots until the sixth week by which time the output from the potted plants had begun to decline. Although there was no difference finally between the root weight of the two types of plant the potted plants grew taller and produced bigger leaves than the water culture plants, the mean heights of haulm being approximately 23 in. and 17-18 in. respectively. The L.A. values from the potted plants (means of two replicates) are plotted against those from the jars for each treatment in Fig. 2.

The data from the plants supplied with fresh nutrient every eighth day showed that over six successive changes the L.A. value achieved the first day after a change, averaged L.A. 1.1. Assuming diffusate production to be relatively constant over the eight days a theoretical line was constructed to represent the expected build up in activity with time. This is shown by the solid line in Fig. 3, for both four

and eight day plants. The L.A. values actually achieved are also given and show good agreement with the theoretical lines within the limits of experimental error which suggests that little breakdown of the diffusate occurred up to eight days.

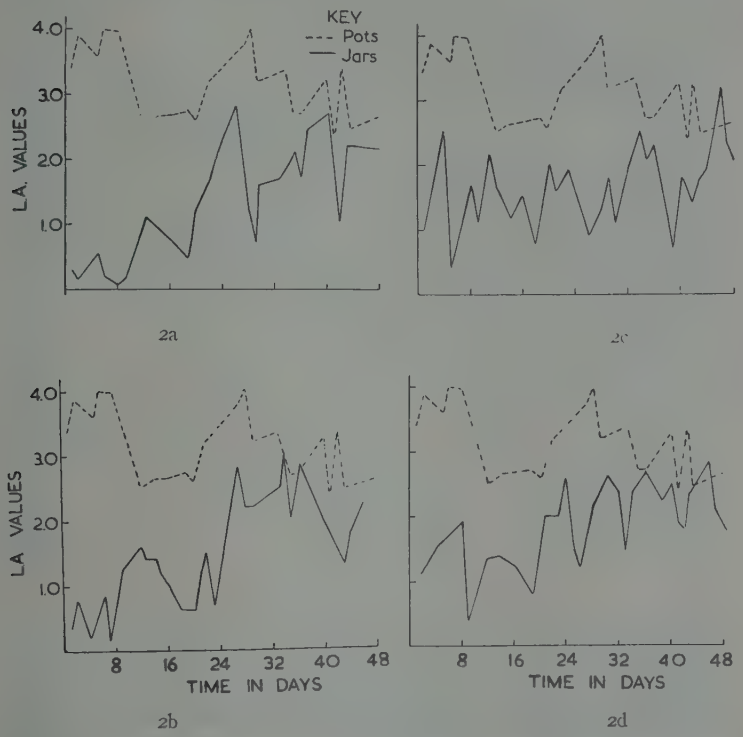

Fig. 2. Showing the diffusate production of potatoes grown in pots and in water culture in expt. 2.

a. Production in pots and in nutrient solution changed daily.
b. Production in pots and in nutrient solution changed every 2 days.
c. Production in pots and in nutrient solution changed every 4 days.
d. Production in pots and in nutrient solution changed every 8 days.

In experiments 1 and 2, the nutrient solution had not markedly increased the output of diffusate and, in the third experiment on the same plan as experiment 2, the plants were grown in distilled water only, but approximately 50 ml. of nutrient solution was sprayed on to the foliage of all the plants including the potted controls every day. Growth under these conditions was vigorous and the roots were com-

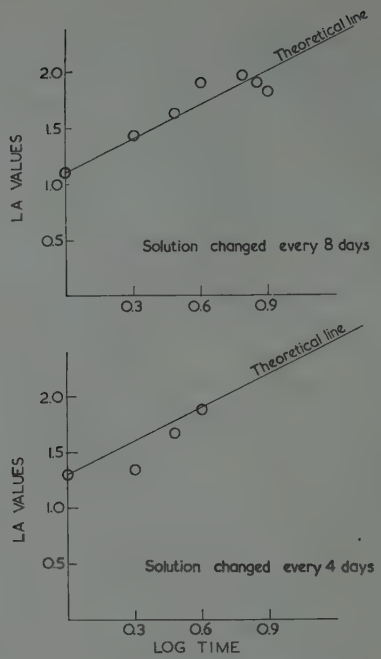

Fig. 3. Showing the observed and expected diffusate output of potatoes in nutrient solution changed every 8 and every 4 days.

parable in weight with those of the previous experiment. However, although the production of diffusate by the potted plants again exceeded that of the water culture plants for the first three weeks it then declined rapidly until six weeks, when the experiment was closed. As before production in the jars was slow initially but rose steadily to a peak of L.A. 3.0 at five weeks from the plants receiving a change of water every day; at this stage the pots gave 1 litre of diffusate of L.A. 2.2 daily. The rate of build up in the undisturbed jars was lower than that expected.

DISCUSSION

The tests showed that diffusate was produced by potatoes growing in one litre of nutrient solution without aeration but that for at least the first four weeks of growth it was less active than that from potatoes in pots, then at their peak of production. This indicates that growth was slow initially in water culture. The accumulation of dif-

fusate in the jars left undisturbed for periods up to eight days approximated to the expected level and did not deteriorate when the plants were grown in nutrient solution. When they were grown in water, the build up was less, possibly reflecting a slower rate of growth. In the later stages of experiments 1 and 3, the daily output of diffusate from one and two day treatments was equivalent to that after eight days standing, suggesting that except in the early stages, there is no value in allowing the diffusate to accumulate, particularly when the plants are growing in water.

The colour of the foliage and the final weight of roots of the water culture plants roughly equalled those of the potted plants in two experiments but the height of haulm was generally lower. The rate of nutrient uptake from the solution was not measured but the daily change of nutrient solution did not appreciably increase growth in experiment 2 and, as no deficiency symptoms developed in the four and eight day treatments, it was concluded that the plants were not starved (HOUGHLAND loc. cit). Possibly a slightly lower pH and aeration of the solution would increase the early rate of growth and improve the height of the plants.

I wish to thank Dr. D. W. Fenwick for many helpful discussions and Mr. Richard Johnson for his assistance in making mounts for the potatoes.

ZUSAMMENFASSUNG

Zur Frage der Produktion von Kartoffelwurzeldiffusat wurden Untersuchungen in Wasserkultur durchgeführt. Die Pflanzen wuchsen in Gefässen mit Nährlösung oder Wasser im Freien bei einer Wurzeltemperatur von 10 bis 16° C und einem pH von 5,5 bis 6,0. Das Volumen der Lösung war begrenzt und auf einer täglichen Vorrat von 1 Liter konzentrierten Diffusats je Pflanze abgestellt. Eingetopfte Kartoffeln produzierten das stärkste Diffusat bis etwa 4 Wochen nach dem Auflaufen, aber die Produktion der Pflanzen aus den Wasserkulturen wird jedoch bis etwa 7 Wochen nach dem Umpflanzen beobachtet. Sie wurden von oben her angemessen ernährt, wobei die Lösung aus „Halna"-Töpfen gewonnen wurde.

Im ersten Versuch wuchs die Hälfte der Pflanzen abwechselnd in Wasser und dann in Nährlösung, die andere Hälfte nur in Wasser. Das Wachstum in allen Versuchsreihen war vergleichbar, aber die Pflanzen, die jeden zweiten Tag zwischen Wasser und Nährlösung wechselten, brachten das aktivste Diffusat. Daher wurden Pflanzen später nur in Nährlösung angezogen. Das Diffusat war weniger aktiv als das in den Kontrolltöpfen bis zu 4 Wochen produzierte, aber es war mit dem von 4 bis 6 Wochen produzierten vergleichbar. Die Gesamt-Ausbeute aus den Gefässen war geringer als die aus den Töpfen.

Im zweiten Test wuchsen Pflanzen in Wasser, und Nährlösung wurde auf die Blätter gespritzt. Die Diffusat-Ausbeute liess sich besser mit der aus den Töpfen vergleichen, die nach 3 Wochen Wachstum sehr schnell abnahm, doch produzier-

ten die Wasserkultur-Kartoffeln wie in früheren Versuchen kein Wurzeldiffusat von mehr als L.A. 3,0 vor 4 Wochen Wachstum, nach welcher Zeit eingetopfte Kartoffeln den Gipfel ihres schnellen Wurzelwachstums und die entsprechende Diffusatabgabe bereits überschritten hatten.

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Received for publication: October 18, 1957

Nematologica III (1958): 115-126. E. J. Brill, Leiden

EFFECT OF OSMOTIC CONCENTRATION ON HATCHING OF SOME PLANT PARASITIC NEMATODES

BY

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The ability of plant parasitic nematodes to survive in soil between successive host plant generations is well known, for example, in *Heterodera* and *Ditylenchus*. However, we know little about the physiological mechanisms that operate in these and other genera in response to environmental stresses. The present study shows that the response of certain nematode eggs to an environmental factor, moisture stress, enables the organisms to survive long periods of drought in the soil.

Our work began with an observation (by G.C.M.) that larvae of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 failed to emerge from eggs kept in dilute fertilizer solutions. This led to a determination of the exact concentration of NaCl necessary to prevent hatching of root-knot nematodes (*Meloidogyne sp.*) and to an examination of the effect of other salts and of dextrose. A detailed study of hatching curves and recovery from inhibition was also made on eggs of *Heterodera rostochiensis* Wollenweber, 1923 in graded concentrations of NaCl and $Ca(NO_3)_2$. A study of the embryology of *M. arenaria* (Neal, 1889) Chitwood, 1949 in water and in salt solution led to speculation on the nature of action of root diffusates on *Heterodera* larvae.

MATERIALS AND METHODS

Individual egg masses of root-knot nematodes were picked by hand from root surfaces and kept in fluid in small dishes. Cysts of golden nematodes (*H. rostochiensis*) were collected from infested soil by flotation. Batches of root diffusate sufficient for an entire experiment were collected on one day from tomato plants and stored at -4 to -5° C. Cysts, eggs and larvae were handled under the dissecting microscope at 15-30 × magnification. In an effort to prevent growth of fungi during prolonged immersion in test solutions we accidentally devised

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a treatment to separate eggs of root-knot nematodes from the mucoid substance in which they are normally embedded. Egg masses were removed from roots immersed in 2 % NaCl and transferred to 3 % H_2O_2 for twenty minutes followed by thorough washing in distilled water and transfer to 1 % NaCl solution. After about one day the eggs were gradually released from the mucoid substance and could be handled individually. Mature females with egg masses removed have been kept alive in water and in NaCl solution for many days. Eggs are deposited in adhering strings in water and singly in NaCl, in both cases without the formation of a second egg sac.

RESPONSE OF *M. javanica* TO SOLUTIONS OF NACL

Experiments conducted at Salisbury, S. Rhodesia, were designed to determine whether the above-mentioned treatment with H_2O_2 altered response of eggs to a graded series of concentrations of NaCl solutions. Five egg masses removed from roots in 2 % NaCl and washed in distilled water were compared at each concentration of test solution with five masses similarly removed in 2 % NaCl and then treated with H_2O_2 followed by washing in distilled water. After the egg masses had been placed in their respective test solutions, three rapid changes of solution were made. Hatched larvae were removed and counted daily at first and then at irregular intervals. Solutions were changed at each count. After 59 days, test solutions were replaced with distilled water and counts made for another 31 days.

Table I presents percentage of total hatch of each series of five egg masses during 59 days in test solutions. In addition, the total number of larvae hatched after an additional 31 days in distilled water is given. Individual egg masses varied greatly both in total number of emerging larvae and in percent hatch in salt solutions. The data indicate a depression of hatch at higher concentrations of NaCl. Immersion for 59 days in NaCl solutions at the concentrations tested did not prevent subsequent emergence in water. Treatment with H_2O_2 did not alter the final number of free larvae. In the control series of eggs, larval emergence was almost complete by the 26th day in water in both treated and untreated series. However, eggs which had been inhibited in higher concentrations of NaCl hatched much more rapidly than this upon transfer to distilled water so that emergence was virtually complete by the 10th day. In this case as well, rates of emergence were not altered by treatment with H_2O_2.

An effort was made to determine how long eggs would remain viable when held in salt solutions. Eggs held in 0.3 molar NaCl for as long as

TABLE I

Larvae hatched from 5 egg masses of Meloidogyne javanica kept in NaCl solutions of various molar concentrations for 59 days and then transferred to distilled water for an additional 31 days

	Molarity of NaCl solutions								
Experiment	*0.0*	*0.17*	*0.19*	*0.21*	*0.22*	*0.24*	*0.26*	*0.27*	*0.29*
I* Percent hatch on day 59 in salt	100	44	4	37	10	11	3	12	4
Total hatch on day 90 in water	3313	1463	3857	2678	1932	2836	1460	1398	2034
II** Percent hatch on day 59 in salt	100	14	27	27	29	2	3	0.8	0.1
Total hatch on day 90 in water	3050	3002	2244	2260	1953	1329	1748	1185	2023

* Experiment I = egg masses dissected in 0.3M NaCl, washed in water, and placed in test solution.

** Experiment II = egg masses dissected in 0.3M NaCl, placed in 3% H_2O_2 for 20 minutes, washed in water, then placed in test solution.

150 days produced larvae upon transfer to distilled water. Counts were made on four series of five masses each, held for 49, 70, 101 and 150 days respectively. Some reduction in number of emerging larvae occurred in eggs held for 150 days in NaCl, and there is a suggestion that emergence was slower after the more prolonged exposures to NaCl solutions.

RESPONSE OF *H. rostochiensis* AND *M. arenaria* TO SOLUTIONS OF HIGH OSMOTIC CONCENTRATIONS

Experiments at Seaford, N.Y., were designed to determine whether inhibition is caused by the osmotic concentration or by particular ions. Five concentrations of NaCl, $CaCl_2$, KCl and dextrose were tested (0,1, 0.2, 0.3, 0.4 and 1.0 molar). A few preliminary tests were also made with Ca $(NO_3)_2$ and NH_4NO_3. Five egg masses of *M. arenaria* were exposed singly to salt or sugar solution at each concentration, with one egg mass in water as a control. Ten cysts of *H. rostochiensis* were placed together in dilute root diffusate and ten cysts together in each test solution. The latter were prepared in such a way as to keep the concentration of root diffusate constant in all solutions.

As the concentration of salt or dextrose rose, hatching was progressively inhibited (Table II). Complete inhibition occurred at lower concentrations of salts than of dextrose. Failure of larvae to hatch could be overcome, even after the most concentrated solutions used, by trans-

ferring eggs or cysts to water. Similar results were obtained in tests with $Ca(NO_3)_2$ and NH_4NO_3.

TABLE II

Inhibition of hatching of larvae of Heterodera rostochiensis (H.r.) and Meloidogyne arenaria (M.a.) in solutions of salts and of dextrose

	Chemicals in Solutions							
	NaCl		KCl		$CaCl_2$		Dextrose *	
Molarity	H.r.	M.a.	H.r.	M.a.	H.r.	M.a.	H.r.	M.a.
0.1	+	—	+	—	+	+	±	—
0.2	++	++	+	++	++	++	+	±
0.3	++	+++	++	++	+++	+++	++	++
0.4	+++	+++	+++	++	+++	+++	++	++
1.0	+++	+++	+++	+++	+++	+++	+++	+++

— = no reduction or delay in hatching
± = doubtful reduction or delay
\+ = some reduction or delay
++ = marked reduction or delay
+++ = complete inhibition of hatching
* = molal solutions.

The rapid recovery from inhibition made by both species of *Meloidogyne* and by *H. rostochiensis* suggested that the larvae within eggs were not harmed by exposure to high osmotic pressures and that development of *Meloidogyne* may have proceeded during the period of inhibition. Accordingly, isolated 2-celled eggs of *M. arenaria* were placed in water kept at 25 ± 2° C. A parallel series in 0.3 molar NaCl was prepared. Eggs from each series were removed for study at 24-hour intervals. Hatching occurred 260 hours after the 2-celled stage. With one exception, development in 0.3 M NaCl was exactly parallel to that in water in both course and time. Larvae developing in eggs kept in water began to move 93 hours after the 2-celled stage; they continued to move with increasing vigor until hatching. No motion was observed at any stage in the NaCl series. After removal from inhibiting solutions to water, motion began and hatching took place.

At 50× magnification, eggs with larvae nearly ready to hatch can be recognized by their distorted shapes. The larva alternates between inactivity and rapid twisting. From time to time it appears to be pushing out against the membrane by a general straightening movement so that large bulges appear at each end of the egg. As the head is applied to the membrane, the stylet is extruded repeatedly to a distance equal to twice the stylet-knob width. At an ambient temperature of 21° C., thrusts were made at a rate of 70-90 per minute. This type of

behavior has been observed in eggs for as long as three hours prior to hatching. Table III presents observations on a single egg. It is not clear whether a larva weakens the egg membrane mechanically or by deposition of an enzyme. In any case, the larva returns its head to one spot repeatedly, a small tear finally appears, and the head bursts forth. The body slowly unfolds as the head advances. The egg collapses about one minute after exit of the tail.

TABLE III

Behavior of a larva of Meloidogyne arenaria during hatching

Behavior	*Time* (minutes)	*Percent of total*
Motion of larva without stylet thrusts	14.6	38
Motion of larva accompanied by stylet thrusts	3.2	8
Thrusts of stylet without larval motion	21.0	54
Stylet applied to approximate position of emergence	17.1	44
Stylet applied to opposite end of egg	7.1	18

Quantitative data on recovery from inhibition were collected on *H. rostochiensis* since all eggs in mature cysts contain fully developed larvae or are empty. Eggs can be removed quickly from cysts and sorted with micropipettes without treatment since they do not adhere to each other. In the first series, test solutions consisted of root diffusate, 8-hydroxyquinoline sulfate at 1×10^{-6} concentration (to control bacteria and molds), and varying concentrations of $Ca(NO_3)_2$. Both control and test solutions contained 20% diffusate. All cysts came from the same population which had been grown under glass and stored in soil at room temperature for more than a year (Cf. FENWICK, 1955).

Dishes were prepared containing 100 eggs each, randomly taken from a group of 25 cysts. A single series, which could be assembled in about five man-hours, consisted of six dishes for each of five concentrations. Two dishes of a series contained diffusate throughout (control); two contained salt solution + diffusate for 14 days, after which salt was replaced by diffusate; and the remaining two dishes had salt + diffusate throughout. Solutions were changed weekly, and the experiment terminated on the 33d day.

Results of this test are presented in Table IV. Inhibition of hatch was doubtful in 0.05M $Ca(NO_3)_2$. In 0.10M concentration, emergence was delayed, but larvae continued to emerge steadily. At concentrations

of 0.15M or more there was virtually no emergence in salt solutions, but when the eggs were transferred to diffusate without salt, emergence again occurred.

TABLE IV

Cumulative hatch of Heterodera rostochiensis (100-egg series) in root diffusate plus various concentrations of $Ca(NO_3)_2$

Concentration of $Ca(NO_3)_2$	Eggs in salt solution continuously		Eggs in salt solution followed by no-salt	
	14-day hatch	*33-day hatch*	*14-day hatch*	*33-day hatch*
			in salt	*in no-salt*
0.5 Molar	26*	80	23*	64
0.1 „	4	45.5	3	78.5
0.15 „	0	7.5	0	73.5
0.20 „	0	0.5	0	58
0.25 „	0	0.0	0	57
0.0 „ (control)	43.5 ± 3.3**	70.5 ± 3.0		

* = mean hatch of two groups of eggs in each concentration.
** = mean ± standard error of mean; ten groups of eggs in control series.

A parallel experiment was prepared using 50 eggs per dish and varying concentrations of NaCl. Table V shows that inhibition was marked in 0.2M concentration and complete in concentrations of 0.3M or higher. There was no depression in total hatch after removal from salt solutions of 0.2M to 0.6M concentrations. But 1.0M NaCl lowered the total hatch obtainable. Figure 1 presents curves comparing numbers of larvae which emerged daily in this experiment. At 0.2M, the peak of hatching occurred on the second day after removal of the eggs from

TABLE V

Cumulative of hatch of Heterodera rostochiensis (50-egg series) in root diffusate plus various concentrations of NaCl

Concentration of NaCl	Eggs in salt solution continuously		Eggs in salt solution followed by no-salt	
	14-day hatch	*37-day hatch*	*14-day hatch*	*37-day hatch*
			in salt	*in no-salt*
0.2 Molar	1*	11.5	1*	37
0,3 „	0	0	0	34.5
0.4 „	0.5	0.5	0.5	32
0.5 „	0	0	1	26.5
0.6 „	0	0	0	32.5
1.0 „	0	0	0	12.5
0.0 „ (Control)	25.5 ± 1.3**	39.5 ± 1.2		

* = mean hatch of two groups of eggs in each concentration.
** = mean hatch ± standard error of mean; 12 groups of eggs in control series which terminated on day 22.

salt + diffusate to diffusate without salt. But at the higher concentrations, hatching was delayed for several days. Similar results were obtained with series of 100 eggs each in $Ca(NO_3)_2$ solutions where the peak shifted from the first day of removal of salt at 0.10 M to the eighth day at 0.20 and 0.25 M.

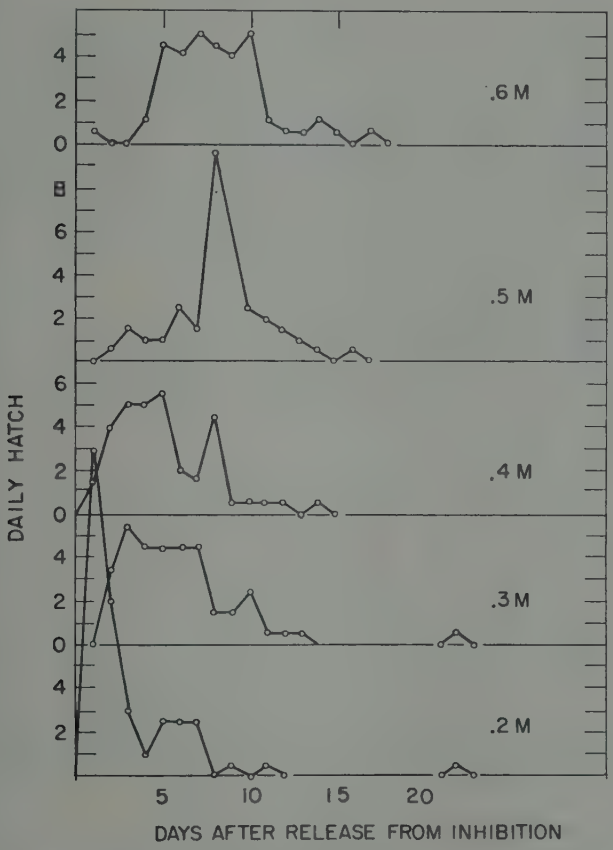

Fig. 1. Effect of concentration of NaCl on rate of hatching of *Heterodera rostochiensis* larvae (50-egg series) after transfer from salt solution to water.

Resumption of hatching was also related to length of exposure to solutions of high osmotic concentration. In the NaCl series, one dish in each dilution contained eggs kept in salt solution for 51 days after which the salt was replaced by control solution. Another dish contained salt solution throughout. Results of this series are presented in

Figure 2. Eggs kept in 0.3, 0.4 and 0.6 M NaCl solutions for 51 days produced larvae after transfer to control solutions more slowly than those held in salt solutions for 14 days. No difference appeared in the 0.5 M set.

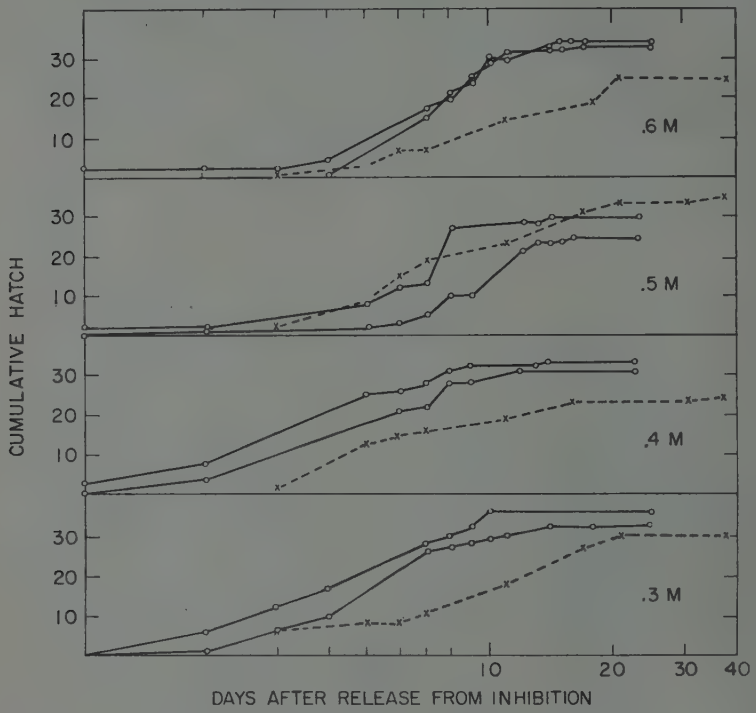

Fig. 2. Effect of time in NaCl solution on cumulative hatch of *Heterodera rostochiensis* larvae (50-egg series) after transfer from salt solution to water.
o———o = 12-14 days in NaCl.
x———x = 51 days in NaCl.

HATCHING OF *Ditylenchus* SP.

In order to determine whether osmotic inhibition of larval emergence is peculiar to the closely related genera *Meloidogyne* and *Heterodera* or is also present in other genera of soil-inhabiting nematodes, a brief test was made with eggs of *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 from daffodils. Eggs were collected by the flotation-centrifugation technique of CAVENESS & JENSEN (1955). Six replications of 50 eggs each in 0.5 M NaCl yielded only three larvae at the end of

nine days, when the salt solution was replaced by water. A control series of 100 eggs had 58 larvae after nine days in water. After the solution was changed, 76 larvae hatched from the 300 eggs which had been in salt solution. This establishes the fact of reversible inhibition in *Ditylenchus*.

DISCUSSION

The question arises whether the inhibition of larval emergence reported here exists also in soil. The maximum concentration of dissolved material in free water in agricultural soil is well below the concentrations found to inhibit hatching. However, soils also hold water by molecular attraction to the surface of soil particles ("soil-moisture tension"). The sum of these two forces is termed "soil-moisture stress" (RICHARDS, 1954). Soil-moisture stress in a given soil depends not only on the total amount of water present, but also on mineral content and physical characteristics. For a given quantity of water the stress is higher in a finely divided soil than in sand. Plant response to this moisture stress has been extensively studied. RICHARDS & WADLEIGH (1952) stated: "Vegetative growth of some plants decreases significantly as soil-moisture stress increases in the one atmosphere range and becomes zero before a soil-moisture stress of 15 atmospheres is attained." The amount of water in soil expressed as percentage of dry weight of soil at just the degree of dryness to cause permanent wilting of seedlings is termed the "wilting coefficient". For most soils, this represents a moisture stress of about 15 atmospheres. Osmotic solutions of this moisture stress in our experiments were 0.3 molar NaCl and 0.2 M Ca $(NO_3)_2$.

WALLACE (1956a) made an extensive survey of the stimulation of hatching of *Heterodera schachtii* by low concentrations of organic and inorganic substances. He noted stimulation at 0.01 molar concentration of various chemicals and inhibition at concentrations above 0.1 molar which he attributed to osmotic pressure. He likewise drew attention to the equality of moisture stress at the permanent wilting point and at the osmotic concentration necessary to stop hatching. LINFORD (1941) buried eggs of root-knot nematodes in soil at various moisture contents. He reported that hatch was delayed or stopped at low moisture, just above the wilting coefficient, but that development was apparently unaffected since hatching occurred very quickly upon restoration of adequate moisture. Growing tomato and cowpea seedlings were invaded by few larvae in soils of low moisture. LINFORD states: "With increasing wetness to just above the moisture equivalent the

intensity of invasion increased. In still wetter soil, it declined."

In our experiments, larvae of root-knot nematodes were infective to tomato plants after eggs had been released from inhibition by 1.0 molar NaCl solution. From our own and from LINFORD's results, it seems that this mechanism permits some nematodes to survive periods of prolonged drought. We have demonstrated inhibition and recovery in one species of *Heterodera,* in two species of *Meloidogyne,* and in a species of *Ditylenchus.* ITO (1955) recorded that eggs of *Schistosoma japonicum* failed to hatch in 1 % NaCl; hatch was rapid and complete when the eggs were returned to water after 96 hours in 1 % NaCl. It is likely that this phenomenon occurs widely among soil-inhabiting nematodes and other helminths.

The relation between soil type and moisture stress may be part of the explanation for the success of certain nematode infections in sandy soils. SLEETH and REYNOLDS (1955) showed that degree of infestation with root-knot in pot tests depended on soil texture. By varying the proportions of loamy sand and clay loam, these workers found that *M. javanica* infections fell as the proportion of finely divided soil rose. Soils differ in many ways in addition to their moisture stress characteristics. WALLACE (1956) suggested that oxygen concentration in soil water is important for larval emergence of the sugar beet nematode. OTEIFA (1955) raised the possibility of specific ion effects on emergence of root-knot nematode larvae.

One puzzling feature of our results has been the length of time necessary for recovery from inhibition by the higher concentrations. In some cases hatch has been delayed for as much as one week. On the other hand, eggs inhibited by lower concentrations of salts recovered very shortly after removal to water. Actively moving larvae within eggs of *M. arenaria* in the final stages of hatching ceased general body motion within a few minutes after a 0.5 molar solution of NaCl replaced the water on the slide. Motion was resumed within five minutes after the substitution of tap water. This effect could be repeated on the same egg. Perhaps eggs inhibited by higher concentrations take up appreciable amounts of the ions in solution so that recovery is delayed until these diffuse out of the system.

It seems useful to view the cyst of *Heterodera* with its contained population of embryonated eggs as an ecological unit (ELLENBY 1956). The same is true for the egg mass of *Meloidogyne.* It is possible that the adult female excretes sufficient material into her egg mass to raise the osmotic concentration of the milieu to an inhibitory level.

Fully developed eggs of root-knot and *Heterodera* larvae offer in-

teresting material for physiological investigations. With the techniques outlined, it is possible to handle individual eggs. The rapid response in rate of larval movement to changes in osmotic concentration suggests that larval movement may be a suitable indicator of response to dissolved gases, to respiratory inhibitors and other chemicals. Eggs of uniform genetic constitution are available in single-larva cultures of *Meloidogyne*. Populations of eggs ready to hatch may be obtained by storage at suitable osmotic concentrations followed by transfer to water. *Heterodera* cysts contain fully embryonated eggs which can be activated by exposure to diffusate (Cf. FENWICK, 1955).

There is a striking parallel between eggs of *Meloidogyne* inhibited by solutions of sufficient osmotic pressure and eggs of *Heterodera* before exposure to diffusate. In both, development has proceeded to completion within the egg, but there is no larval motion. When the osmotic concentration of the medium is lowered, a *Meloidogyne* larva begins to move and hatching ensues: some *Heterodera* larvae require a stimulus, usually supplied by a substance in root diffusates from growing host plants, for activation and emergence. One possibility is that in both cases absence of water is the critical factor and that the diffusate alters egg or larval permeability to permit passage of water and of other molecules and ions. Under this assumption, *Meloidogyne* eggs not requiring a diffusate would be expected to have higher permeabilities than eggs of *Heterodera*. The well-known stimulation of hatching by a variety of inorganic and organic substances in great dilution would result from the influence of such stimulants on the movement of solutes across egg and larval membranes. There are, of course, other possibilities, such as the entry of the diffusate directly into metabolic processes. At present, no choice has been made between these possibilities.

ZUSAMMENFASSUNG

Die Beobachtung, dass Larven von *Meloidogyne javanica* nicht aus Eiern schlüpfen, die in verdünnten Handelsdüngerlösungen aufgehoben wurden, führte zu einer Reihe von Versuchen über die Wirkung von Lösungen verschiedener osmotischer Drucke. In einleitenden Versuchen wurden Eier von Wurzelgallennematoden durch Behandlung mit Wasserstoffsuperoxyd und Natriumchlorid voneinander getrennt. Diese Behandlung hat keinen Einfluss auf das nachfolgende Verhalten der Larven in den Testlösungen. Das Ausschlüpfen wurde durch Lösungen von etwa 0,3 mol. Natriumchlorid verhindert. Beim Überführen in Aqua dest. entwickelten sich aus den Eiern Larven, selbst noch nach einem 150 tägigen Aufenthalt in Salzlösung.

Ähnliche Ergebnisse wurden in Versuchen mit fünf Konzentrationen von Natriumchlorid, Kaliumchlorid, Calciumchlorid und Dextrose auf Eier von *M. arenaria* und Cysten von *Heterodera rostochiensis* erhalten. Die Schlüpfhemmung

konnte selbst nach 1,0 mol. Lösungen durch Übertragen der Eier oder Cysten in Wasser überwunden werden.

Die Entwicklung von *M. arenaria* schreitet in 0,3 mol. Natriumchlorid normal weiter, ausser wenn Eier bei 25° C in Wasser gehalten worden waren und die Larven sich 93 Stunden nach dem Zweizellenstadium zu bewegen begannen. Larven zeigten niemals Bewegung, wenn die Eier in 0,3 mol. Natriumchlorid gehalten worden waren. Der Schlüpfprozess in Wasser wird im einzelnen beschrieben.

Eine Reihe von Versuchen mit Eiern von *H. rostochiensis* in Lösungen von Calciumnitrat und Natriumchlorid zeigte, dass die Erholung den osmotischen Konzentrationen der schlüpfhemmenden Lösung und der Zeitdauer umgekehrt proportional war. Larven von *M. arenaria* befielen Tomatenpflanzen, nachdem die Eier einer 1,0 mol. Natriumchloridlösung ausgesetzt worden waren. Eier von *Ditylenchus dipsaci* aus Narzissen zeigten dieselbe Reaktion wie die von *Meloidogyne* und *Heterodera.*

Zwischen diesen Ergebnissen und den Fällen, in denen die Bodenfeuchtigkeit nicht allein vom osmotischen Druck, sondern auch von dem molekularen Haften des Wassers an der Oberfläche der Bodenteilchen abhängt, wird eine Parallele gezogen. Da bei einer Bodenfeuchtigkeit, die einer Saugkraft von 15 Atmosphären entspricht, die Pflanzen allgemein welken, scheint es, dass der in dieser Arbeit aufgezeigte Mechanismus es einigen Nematoden erlaubt, Trockenheitsperioden zu überleben. Eine weitere Parallele zwischen den Eiern von *Meloidogyne*, die durch Lösungen genügender osmotischer Konzentration in ihrem Schlüpfen behindert sind und den Eiern bestimmter *Heterodera*-Arten vor dem Zusetzen eines Wurzeldiffusats lässt vermuten, dass das Diffusat die Permeabilität der Eier und Larven verändern kann und dadurch den Eintritt von Wasser und gelösten Substanzen ermöglicht.

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Received for publication: November 12, 1957

Nematologica III (1958): 127-135. E. J. Brill, Leiden

EXPERIMENTAL METHODS IN TESTING FOR RESISTANCE TO BEET EELWORM, *HETERODERA SCHACHTII* SCHMIDT

BY

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In view of the difficulties involved in attempting to control eelworm by chemical methods, it is important to consider breeding for resistance to this pest. With the potato root eelworm, *Heterodera rostochiensis* Woll. some progress has been made in breeding resistant potatoes on a field scale, using wild species having a high degree of resistance in crosses with commercial varieties (HUYSMAN, 1957).

In 1935, HÜLSENBERG began breeding sugar beet for resistance to the beet eelworm, *H. schachtii*, following up some early work by MOLZ (1917) and HUSFELD (1926). HÜLSENBERG tried to select both for tolerance to attack and a reduction in the number of cysts produced, making his selections from commercial and inbred lines.

More recently, Dutch workers have been studying this problem on a more intensive scale. HIJNER (1951) tested various species of beet and found that three were resistant [2]). These were the members of the group Patellares within the genus *Beta*, which consists of the species *B. patellaris* Moq., *B. webbiana* Moq. and *B. procumbens* Chr. Sm. Other species, including *B. vulgaris* ssp *maritima* (L.) Thell. and commercial varieties of *B. vulgaris* L. were found to be highly susceptible. However, attempts to introduce resistance into sugar beet have so far met with no success, since the F_1 progeny from crosses with species of the Patellares group are very hard to raise. Where germination of F_1 seed is obtained, the seedling dies at an early stage.

Because of these difficulties, it was thought advisable to look for resistant or partially resistant individuals or lines of commercial sugar beet, the wild species *B. maritima*, and crosses between these two. This course has been adopted in Holland (RIETBERG, 1954) and more recently in America (MCFARLANE, MORGAN GOLDEN, *in litt.*), where

1) Now at Rothamsted Experimental Station, Harpenden, Herts.

2) The term "resistance" applies here and throughout this paper to plants in which female eelworms fail to develop, or are much reduced in numbers.

previously emphasis had been on breeding for tolerance to attack (THORNE *et al.*, 1938; SWINK, 1954; FINKNER & SWINK, 1955, 1956). In both these countries screening of plants is carried out under glass. Before commencing a similar screening programme in England, experiments were made on the suitability and reliability of techniques.

VARIABILITY ARISING IN DIFFERENT METHODS OF TESTING

Nothing was previously known of the variation in the degree of attack occurring amongst beet of the same line under standard conditions, or of the effect of the methods used and seasonal conditions on that variation.

In the late autumn of 1955, three methods of producing an infestation of beet plants grown in small pots ($2^1/_2''$) were tried. About 25 plants were used for each method, seed from a single source of a well-known commercial variety of sugar beet being used throughout. The seed was sown in seed boxes and transplanted at the cotyledon stage into the pots.

The three methods consisted of (1) the addition of hatched larvae at the rate of 2,000 per pot of potting compost (i.e. at the rate of 20 larvae/gm. soil) (2) the addition of 50 hand-selected cysts per pot (estimated to give 20 larvae/gm. soil), using the same compost and (3) dilution of infested field soil with compost to approximately the same infestation level. In the first method, the larvae were added to each pot two weeks after the seedlings were transplanted. Between one and two weeks after the first appearance of cysts the number of white cysts on the peripheral root system was counted, using a binocular microscope, and also the total number of new cysts per pot were later estimated by elutriating the preserved contents of the pot (FENWICK & REID, 1951). An analysis of the results is shown in Table I.

In the summer of 1956, a further experiment was carried out using two levels of infestation with hatched larvae, at the rate of 20 and 40 larvae/gm. soil, i.e. 2,000 and 4,000 larvae per pot respectively. These results are also included in Table I.

The following points of interest arise from the results:

1. The range of cyst counts is very large in every experiment.
2. The total number of cysts developed per plant is much higher under summer than under winter conditions.
3. In the small pots used, the higher rate of inoculation does not result in an increase in the mean number of cysts developed.

TABLE I

Analysis of results of cyst counts showing the effect of season and of various methods of infestation

Exp. No.		No. of Plants	Outside root count of cysts				Total cyst count			
			No. cysts/plant Range	Mean No. cysts/plant	Standard deviation	Coefficient of variation	No. cysts/plant Range	Mean No. cysts/plant	Standard deviation	Coefficient of variation
Winter	I	25	1-200	39	42	109 %	19-380	154	71	46 %
	II	23	2-115	46	33	71 %	10-150	67	42	63 %
	III	25	0-210	67	66	99 %	23-297	115	75	65 %
Summer	IV	25	10-188	86	45	52 %	50-965	644	219	34 %
	V	25	3-91	30	22	77 %	56-750	347	177	51 %

KEY: *Winter*

I 2,000 larvae per pot in potting soil (20 larvae/gm soil).
II 50 cysts per pot in potting soil (20 larvae/gm soil).
III Infested field soil with approx. 50 cysts per pot.

Summer

IV 2,000 larvae per pot (20 larvae/gm soil).
V 4,000 larvae per pot (40 larvae/gm soil).

4. The coefficients of variation of the outside root count of cysts are very high.
5. A further analysis of the coefficients of variation for total cyst counts given in Table I has shown that the differences between them are not significant.

The correlation between outside root counts of cysts and total cyst counts is expressed as correlation coefficients in Table II. It can be seen that, as might be expected, there is a strong correlation between the two, but care must be exercised in applying this to the use of the outside root count as an estimate of the total cyst count. This is because, as a sampling method, it is unusual in that only one sample is available and this sample is not truly representative of the population because of its fixed position relative to the plant. Also, as already referred to above, the coefficient of variation for outside root counts is very high indeed. Therefore, unless a very high rate of replication is used, which is often impracticable, an outside root count of cysts will not detect relatively small differences in the number of cysts which plants of any one line will support. However, by selecting only those plants which have a very low outside root count, it may prove possible to detect resistance in individuals, although, again because of the high variation, a single test is not regarded as reliable. All plants must be subjected to at least one further test.

TABLE II

Correlation between outside root counts of cysts and total cyst counts, expressed as correlation coefficients

Exp. No.	Correlation Coefficient	n-2	Significance
I	.8854	23	Significant at 0.1 %
II	.6964	21	" " "
III	.7602	23	" " "
IV	.5766	23	" " "
V	.3395	23	Insignificant

It may be that where inoculation with cysts is used, the period over which larvae are freed into the soil is protracted. This might account for the lower means in the experiments where this method is employed, because invasion and development would also be protracted as a consequence. The addition of hatched larvae to the soil means that they can invade almost simultaneously and that therefore they develop more

or less concurrently. This is an advantage since, for practical purposes, where the outside root count is used as an estimate of attack, the cysts must be counted during the relatively short period that they remain white.

TECHNIQUE AT PRESENT IN USE

Because the method of adding hatched larvae to the seedlings is convenient, and has been shown to be at least as reliable as that of adding cysts, it has been adapted for use in large-scale testing of material for resistance under greenhouse conditions as follows:

Seed is sown in boxes of sterilised potting compost and transplanted at the cotyledon stage into $2^1/_2''$ pots containing the same compost. Two weeks after transplanting, 2,000 larvae of beet eelworm are added to each pot, giving an infestation level of 20 larvae per gm. soil. The larvae are obtained from a stock of soil from a single source, infested with a population of beet eelworm cysts at a known level. The cysts are floated off from the soil as needed, using conical flasks, as described by WILLIAMS & WINSLOW (1955). The float, which consists of cysts plus organic debris, is spread out on small sieves of nylon material of about 80 meshes/inch, these sieves fitting into petri dishes. About 20 ccs of beet root diffusate plus 2 ccs 10 ppm. mercuric chloride are added to each dish so that the level of the liquid is just up to the nylon of the sieve. In this way the cysts are suspended on the surface of the diffusate and are well aerated. The cysts are kept at 25° C. for a week, the larvae falling to the bottom of the petri dish as they hatch. The larvae are always used at the end of one week and the number of cysts floated is adjusted to give the required number of larvae at the end of the weekly period for the number of plants to be inoculated. The cysts are easily removed on the sieve, and the larvae estimated by using the WINSLOW counting chamber. The suspension of larvae is then diluted to give 1,000 ± 100 per cc. The suspension is then kept agitated by means of a vibratory stirrer *), and 2 cc aliquots removed with an automatic pipette made from a 5 cc hypodermic syringe. A piece of rubber tubing firmly affixed to the spindle of the plunger of the syringe stops the intake at 2 ccs. It is found that this is a convenient volume of liquid in which to add the larvae to each pot. Afterwards, the larvae are gently watered into the pots with a small

*) Vibro-mischer — Dr. Ing. Hans Müller and AG. für Chemie-Apparatbau, Zürich.

quantity of water. This whole process is very rapid. For 120 plants, the cyst flotation takes about an hour, with the soil at present in use. The collection and estimation of larvae and adjustment of the suspension takes approximately one hour and inoculation of pots ten minutes. The apparatus used is shown in Fig. 1. Where possible, thirty plants of each line of beet are tested.

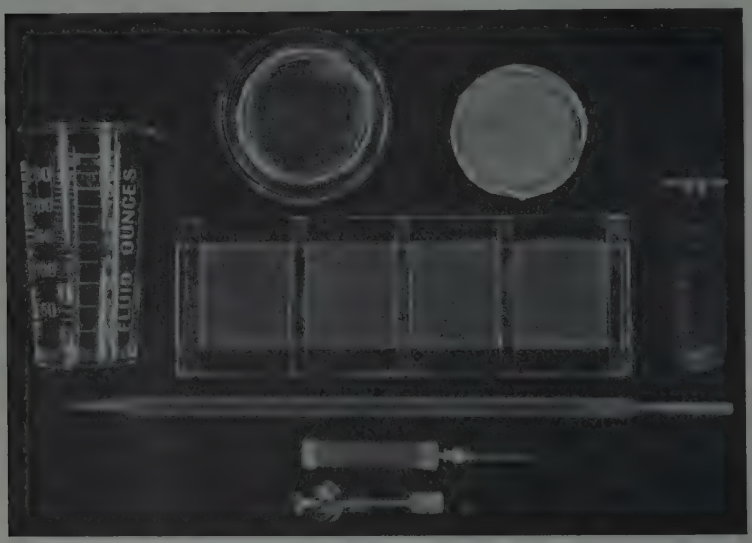

Fig. 1. Apparatus used for hatching larvae from cysts and adding them to pots; sieve and petri-dish for hatching larvae; graduated beaker to hold suspension; pipette, graduated tube and Winslow counting slide for estimating the suspension; adapted hypodermic syringe for adding larvae to pots.

After inoculation, the plants are left until white cysts first appear on the peripheral root system. Two to three weeks later, according to season, the plants are scored by eye for number of cysts on the peripheral root system and also for size of root system. Records are kept of each plant tested. Only those plants with less than ten cysts on the peripheral root system are retained. The whole root system of these plants is then examined and if the cyst density is low over the whole, it is re-planted in a $2^1/_2''$ pot and the treatment immediately repeated. The same criteria are used, and any plant which satisfies them on the second occasion is saved for seed. This technique has been used throughout the summer of 1957, and about 1,500 plants have

been tested. It had been hoped to continue testing material throughout the winter months also, with the aid of heating and lighting equipment, but experience has shown that winter results are unreliable. It is hoped to improve conditions so that winter testing will be possible.

RESULTS

The material tested this year has been mostly inbred lines of sugar beet and crosses between sugar beet and *Beta maritima*. These have been obtained from the Plant Breeding Institute, Trumpington, where they have been selected in connection with virus yellows resistance. This does not imply that there is thought to be any correlation between resistance to virus yellows and to eelworm. The lines used have been selected at random as a starting point for eelworm resistance tests.

By the end of the present testing season, 53 lines will have been fully tested. Up to the present, 1,540 plants have undergone the first test, 160 of which were selected for retesting, representing 34 lines. Of the 96 plants which have completed the second test so far, only 12, representing 5 lines, have been selected for seed production. It will not be known whether any of this material is truly resistant until the progeny has been tested.

DISCUSSION

The limitations of such a greenhouse test are clearly recognised. However, if these limitations are taken into account, the method provides a basis for the selection of likely individuals and allows the testing of a much larger range of material than would be possible under field conditions. Any material selected from the greenhouse test can then be tested in microplots under field conditions with known eelworm population levels, and its reaction to these levels and also its effect upon them can be estimated.

In future testing for resistance, the possible existence of resistance-breaking biotypes of beet eelworm must be considered. Such biotypes have already been found amongst populations of potato-root eelworm (Dunnett, 1957; Jones, 1957) and various populations of beet eelworm from different parts of England are at present being examined at Cambridge, using *Beta patellaris* as test material.

Breeding for beet which can be grown without increasing the population of beet eelworm in the soil, is, of course, not the only important aspect to be considered. Because of the nature of so-called resistance in cyst-forming nematodes, where invasion and partial development

frequently occurs (JONES, 1954), the resistant plant, if grown under a high infestation, is liable to show the same symptoms as the true host plant. Indeed, in some cases, for example in the wild potato, *Solanum tuberosum* ssp. *andigena* Juz. & Buk. (JONES, 1954) and the wild beet, *Beta patellaris* (SHEPHERD, 1957), development extends to the production of males, and heavy infestation may have a marked effect on these plants. It is, therefore, essential to select for tolerance to attack in conjunction with a low cyst production. This can be carried out by selection in the field, as has been done in the U.S.A., or by incorporating in the greenhouse test the weighing of infested and control plants of all the lines tested for resistance.

The very high variation encountered in pot tests necessitates a careful planning of experiments and caution in interpretation of results when such a technique is used. Also, if conclusions are to be based on sampling methods, such as counts of cysts on the peripheral root system of infested plants grown in pots, replication must be of a very high order before the reliability of the results can be considered to be beyond question.

I should like to thank Mr. F. G. W. Jones for his initiation of this work and for his interest in it. I am also grateful to Drs. F. M. Moriarty and R. A. Dunning for help in the preparation of this paper; to Dr J. W. Seinhorst for suggestions on techniques; to Messrs. W. R. Ridgman and J. D. Whitear for advice on statistics; and to Miss M. Wolfe for technical assistance. This work has been financed by the Sugar Beet Research and Education Committee.

ZUSAMMENFASSUNG

Nach einem kurzen Rückblick über frühere Arbeiten zur Resistenzprüfung gegenüber Rübennematoden werden Versuche beschrieben, um die Variabilität aufzuzeigen, die bei der technischen Durchführung solcher Versuche eintritt. In Topfversuchen mit Zuckerrüben ist die Abweichung in der Cystenzahl, die sich an Pflanzen der gleichen Linie bilden, ohne Rücksicht auf die benutzte Infektionsmethode sehr hoch. Sie richtet sich nach der Zahl der Cysten an der Peripherie des Wurzelsystems und der Gesamtcystenzahl je Pflanze. Auch jahreszeitliche Einflüsse beeinflussen die Cystenzahl trotz Benutzung zusätzlicher Erwärmung und Belichtung.

Die für die Durchführung von Routine-Untersuchungen auf Resistenz gegenüber Rübennematoden benutzte Technik, die auch die Zugabe von 2000 geschlüpften Larven zu jedem Rübensämling in 6 cm-Töpfe einschliesst, wird im einzelnen beschrieben. Dreissig Pflanzen jeder Linie wurden, soweit möglich, getestet. Die Bewertung nach der Grösse des peripheren Wurzelsystems und nach der Zahl der weissen Cysten wurde zwei bis drei Wochen nach dem ersten Auftreten weisser Cysten durchgeführt. Nur solche Pflanzen, die weniger als 10 Cysten an der Aussenseite des Wurzelsystems hatten, wurden zurückbehalten.

Diese wurden ein zweites Mal in gleicher Weise geprüft und nur solche für die Samenproduktion benutzt, die wiederum weniger als 10 Cysten hatten. In diesem Jahr wurden 1540 Pflanzen aus 53 Linien geprüft und 160 Pflanzen aus 33 Linien für die nochmalige Prüfung ausgewählt. Gegenwärtig sind 12 Pflanzen aus 5 Linien für die Samenproduktion ausgewählt worden.

Schliesslich werden andere Gesichtspunkte, die bei dem Selektionieren auf Resistenz beachtet werden müssen, z.B. die Möglichkeit des Auftretens resistenzbrechender Biotypen des Rübennematoden und die Selektion auf Toleranz, diskutiert. Auch wird wegen der grossen Abweichungen, die in den Topfversuchen auftreten, die Notwendigkeit sorgfältiger Planung der Versuche und Vorsicht in der Auswertung der Ergebnisse bei Verwendung der erwähnten Technik herausgestellt.

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Received for publication: October 29, 1957

Nematologica III (1958): 136-139. E. J. Brill, Leiden

THE TAXONOMIC POSITION OF *ANGUILLULINA OBTUSA* GOODEY, 1932 AND 1940

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T. GOODEY (1932) described and figured a female nematode which he designated *Anguillulina obtusa* (Bastian, 1865), believing it identical with Bastian's *Tylenchus obtusus*. He regarded it as close to *A. pratensis* (now *Pratylenchus*) in head and stylet characters, though resembling *A. dubia* (now *Tylenchorhynchus*) and *A. robusta* (now *Rotylenchus*) in being didelphic. Later he described and figured the male and amplified his description of the female (GOODEY, 1940), eventually placing it doubtfully in the genus *Pratylenchus* (GOODEY, 1951). Meanwhile FILIPJEV (1936) had transferred Bastian's *Tylenchus obtusus* to the genus *Rotylenchus*. FILIPJEV & SCHUURMANS STEKHOVEN (1941) followed suit, reproducing Goodey's 1932 figures, but a note of caution was added, presumably by Schuurmans Stekhoven, who thought that the oesophageal base, as figured by Goodey, suggested affinity with the genus *Tylenchorhynchus* rather than *Rotylenchus*. THORNE (1949) split the Tylenchidae into three subfamilies, placing *Tylenchorhynchus* in the Tylenchinae, *Rotylenchus* in the Hoplolaiminae, and *Pratylenchus* in the Pratylenchinae. At the same time he created the genus *Radopholus*, within the Pratylenchinae, to receive "those didelphic species which most closely resemble nemas of the genus *Pratylenchus*". J. B. GOODEY & FRANKLIN (1956) followed Filipjev & Schuurmans Stekhoven in considering the nematode described by T. Goodey as *Rotylenchus obtusus*.

Interest in the subject has been revived recently by the finding of nematodes obviously belonging to Goodey's "*A. obtusa*", in aqueous extracts from Rothamsted turf. The general proportions, and particularly the shape of the tail in both male and female, are as figured by Goodey. The head (Fig. 1) usually has 3-4 annules (2-3 striations). The arrangement of the oesophageal glands is essentially as shown by GOODEY (1932); it appears to take the form of a "wrap-round" over-

lap, completely or almost completely enclosing the alimentary canal. An interesting variant frequently seen by the writer is shown in Fig. 2. Here a small bulbous structure appears to link the oesophagus and intestine. Occasionally the glands appear less united, forming more

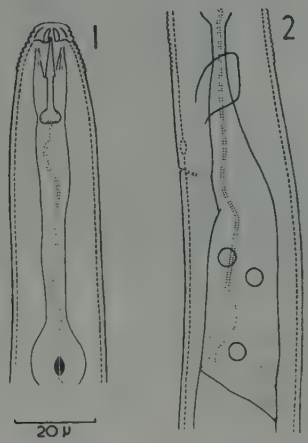

Fig. 1-2. *Pratylenchoides crenicauda* n.sp.
1. Anterior region, showing head shape and annulation.
2. Oesophageal region, showing arrangement of glands frequently encountered by writer.

or less distinct lobes. Otherwise the nematodes appear as described and figured by Goodey. When at rest or killed by gentle heat they usually assume a straight or slightly bent posture reminiscent of *Pratylenchus,* the male tail often curved dorsally as illustrated by Goodey (1940). None of the worms killed by the writer assumed the spiral or open "C" curvature usually associated with *Rotylenchus* and *Tylenchorhynchus* species.

The writer agrees with Sher & Allen (1953), that Bastian's *Tylenchus obtusus* is insufficiently described and illustrated to determine its generic status. Similarly, the data are too meagre to synonymize this species and Goodey's "*A. obtusa*"; consequently the latter requires a new specific name. On head and stylet characters, length and shape of tail in both sexes, and posture when killed by gentle heat, Goodey's nematode is obviously close to *Pratylenchus,* although this genus contains only monodelphic species. It would appear logical, therefore, to place Goodey's nematode in the genus *Radopholus* Thorne. In all known

species of *Radopholus,* however, the oesophageal glands take the form of a long overlap parallel with the anterior region of the gut, contrasting with the shorter oblique overlap of *Pratylenchus* (Sher & Allen, 1953) and "*A. obtusa*". It is therefore proposed to erect a new genus, *Pratylenchoides,* within the Tylenchidae-Pratylenchinae, with Goodey's nematode as the type and sole known representative under the new specific name *crenicauda,* descriptive of the crenate appearance of the tip of the female tail which is usually as shown by Goodey (1932).

Pratylenchoides, n. gen.

Tylenchidae — Pratylenchinae. Close to *Pratylenchus,* but didelphic. Differing from *Radopholus* in the length of the oesophageal overlap which is short and oblique in *Pratylenchoides* and *Pratylenchus,* longer and parallel-sided in *Radopholus.*

Pratylenchoides crenicauda, n. sp.

Synonyms: *Anguillulina obtusa,* Goodey, 1932 and 1940.
Rotylenchus obtusus, Filipjev & Schuurmans Stekhoven, 1941.
nec *Tylenchus obtusus* Bastian, 1865.

Specimens measured by the writer had the following dimensions:
Female (24 specimens): Length = 0.72 mm. (0.57-0.91), a = 26.3 (19-32), b = 4.6 (3.3-6.4), c = 15.3 (12.9-17.6), V = 57.5% (54-61)
Male (6 specimens): Length = 0.69 mm. (0.64-0.74), a = 28.0 (26-33), b = 5.1 (4.5-6.2), c = 13.0 (11.7-15.0)
Head usually with 3-4 annules. Posterior glandular region of oesophagus a "wrap-round" overlap as described by Goodey (1932) and above. Description otherwise as given by Goodey (1932 and 1940).

Thanks are due to Miss V. Ward for technical assistance and help in preparing the figure.

ZUSAMMENFASSUNG

Aus einem Rasenstück bei Rothamsted wurden Nematoden erhalten, die mit *Anguillulina obtusa* Goodey, 1932 und 1940 deutlich identifiziert werden konnten. Es liegen aber nur sehr unzulängliche Beweise für eine Identität mit *Tylenchus obtusus* Bastian, 1865 vor, folglich ist der spezifische Name "*obtusa*" ungültig. Goodey's Art steht der Gattung *Pratylenchus* sehr nahe, ist aber didelphisch. Sie wird als Typ einer neuen Gattung *Pratylenchoides* aufgestellt und hat nach dem Schwanz des Weibchens den spezifischen Namen *crenicauda* erhalten.

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Received for publication: November 25, 1957

Nematologica III (1958): 140-142. E. J. Brill, Leiden

BEOBACHTUNGEN ZUR STACHELLOSIGKEIT DER MÄNNCHEN VON *HEMICYCLIOPHORA TYPICA* DE MAN, 1921 (CRICONEMATIDAE)

VON

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Bei der Untersuchung mitteldeutscher Salzstellen auf freilebende Nematoden fand ich an 2 Stellen *Hemicycliophora typica* de Man, 1921. Neben vollreifen Weibchen und Männchen wurden vor allen Dingen zahlreiche Larven festgestellt, die es mir ermöglichen, etwas über die Häutungsvorgänge dieser interessanten Art auszusagen. Die Fundstellen im mitteldeutschen Raum sind die Salzwiese zwischen Langenbogen und Rollsdorf, wo die Art bei einem Chloridgehalt des Bodens von 3,64 $^0/_{00}$ vorkam, und die Salzwiese von Artern mit einem Chloridgehalt von 9,926 $^0/_{00}$. Beide Fundorte zeigen einen typischen Besatz mit Halophyten, der folgender Zusammenstellung entnommen werden kann (Tabelle I).

TABELLE I

Pflanzenart	Langenbogen	Artern
Triglochin maritimum L.	+	
Phragmites communis TRIN.	+	
Puccinellia distans (JACQUIN) PARL.	+	
Juncus gerardii LOISEL.	+	+
Suaeda maritima (L.) DUM.		+
Salicornia europaea L.		+
Obione pedunculata (GRUFB.) MOQ.		+
Spergularia marginata (DC.) KITTEL	+	
Glaux maritima L.	+	
Plantago maritima L.	+	
Aster tripolium L.	+	+

Über das Vorkommen von *Hemicycliophora typica* in der Salzwiese von Artern wurde bereits von MEYL (1955) berichtet. Der Wiederfund dieser Art in der Salzwiese von Langenbogen zeigt, dass *Hemicycliophora typica* zu den haloresistenten Süsswasserarten zu rechnen ist.

D. PAETZOLD: *Hemicycliophora typica.*

Abb. 1. *Hemicycliophora typica* Weibchen, Vorderende, lateral.

Abb. 2. Weibchen, Hinterende, lateral.

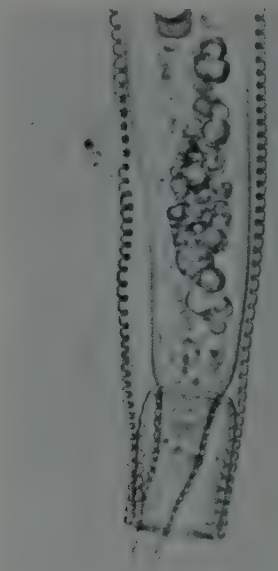

Abb. 3. Larve bei der Häutung. Hinterende, lateral.

Abb. 4. „Männliche Larve" bei der Häutung, Vorderende, lateral.

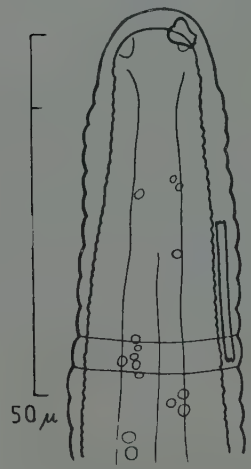

Abb. 5. „Männliche Larve" bei der Häutung, Vorderende, lateral.

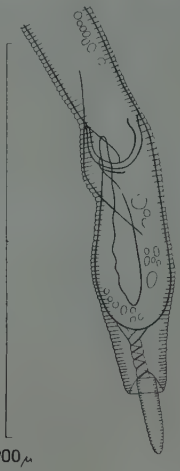

Abb. 6. „Männliche Larve" bei der Häutung. Hinterende, lateral.

Zunächst seien die Masse meiner vollreifen Exemplare (Abb. 1 und 2) angeführt.

	♀ n = 10			♂ n = 3		
L:	1,1	—	1,32 mm	0,745	—	1,043 mm
a:	20,3	—	24,3	26	—	33,4
b:	5,82	—	6,85	5,65	—	6,2
c:	8,04	—	11,1	5	—	5,35
V:	82,5	—	86,2%			
Ei:	110-120,5 : 32,7-33,8 μ					
Sta:	86,5-92,5 μ					

Der eingehenden Beschreibung von MEYL (1955b) und der Wiederbeschreibung von THORNE (1955) habe ich nichts hinzuzufügen. Interessant ist, dass meine Tiere noch etwas grösser sind als die von MEYL und THORNE beschriebenen. Vergleicht man die Masse der bisher beschriebenen Exemplare, vorausgesetzt, dass es sich in jedem Fall um vollreife Tiere gehandelt hat, so lässt sich doch eine recht grosse Variationsbreite feststellen.

	♀		♂	
MICOLETZKY	1925	0,70 — 0,92 mm	DE MAN 1921	0,68 mm
LOOS	1948	0,68 — 0,72 mm	LOOS 1948	0,47 — 0,65 mm
MEYL	1955	1,02 — 1,29 mm	MEYL 1955	0,793— 0,978 mm
THORNE	1955	1,2 mm	THORNE 1955	1,0 mm
PAETZOLD		1,1 — 1,32 mm	PAETZOLD	0,945— 1,043 mm

Unter den zahlreichen Larven (Abb. 3-6) fand ich auch „larvale Männchen" kurz vor Abwerfen der Larvenhaut. Bei der letzten Häutung wird — wie MEYL (1955) richtig vermutete — der Mundstachel abgeworfen. Unter der Larvalkutikula sieht man das stachellose Vorderende des Männchens mit dem zurückgebildeten Ösophagus (Abb. 4 und 5). Beim Vorgang der Häutung scheinen vom Männchen in der Larvalhaut oft mehrere Drehungen durchgeführt zu werden, bei denen der Mundstachel, der sich zwischen Larvalhaut und dem Vorderende des Männchens befindet, in mehrere Stücke zerbrechen kann. Abb. 6 zeigt das Hinterende mit dem umgeschlagenen Schwanz des Männchens und Abb. 5 das Vorderende mit dem zerbrochenen Mundstachel. Stachellose Männchen mit reduziertem Ösophagus sind auch sonst bei stacheltragenden Arten bekannt, z.B. von *Cacopaurus pestis* THORNE, 1943 und von *Iotonchium cephalostrictum* MEYL, 1954. Auf Grund

der bei *Hemicycliophora typica* gefundenen Verhältnisse kann auf eine gleiche Entwicklung bei den letztgenannten Arten geschlossen werden.

SUMMARY

Hemicycliophora typica de Man, 1921 was found during investigation of salt-pits in central Germany near Langenbogen and Artern. Large numbers of larvae were found which made it possible to establish that, in the male, the well-developed larval spear is cast off with the last larval skin so that in adult males the spear is lacking.

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Eingegangen: 24. Dezember 1957

Nematologica III (1958): 143-148. E. J. Brill, Leiden

A TECHNIQUE FOR SCREENING ANTIBIOTICS AGAINST EELWORMS

BY

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Tarjan and Cheo (1956) described several test methods used in the evaluation of chemicals as nematicides. They included a direct toxicity test, with *Panagrellus redivivus* as a test organism, in which the nematicidal value of a chemical was estimated by the time required for the chemical to produce complete inactivation of the eelworm. *P. redivivus* is not a plant parasitic nematode, moreover their's was test *in vitro* and could take no account of any effect the chemical might have on the plant invasion, development and reproduction of the eelworm. The following technique has been designed to test the nematicidal value of antibiotics against the rootknot eelworm, *Meloidogyne incognita* var. *acrita* Chitwood. In it the effectiveness of the antibiotic was measured by observing first whether the treated eelworms were still able to penetrate the roots and secondly whether, if penetration occurred, there was normal development of the eelworm with the production of viable eggs from which larvae also capable of penetrating the roots emerged.

METHODS

Application of antibiotic to the eelworms

The antibiotic broth was applied to suspensions of the infective larvae in deep well-glasses. The larvae had emerged during a 24-hr period from egg sacs in water at 24° C. Batches of 100 larvae in 1 ml of water were put into the glasses by Peters' dilution technique (Peters, 1952). For each antibiotic to be tested there were four batches of eelworms. Four further batches served as water controls. The eelworms from two of these were applied directly to the tomato plants but the other two were spun down in a centrifuge as were those with the antibiotics in the manner described below. This was to test whether centrifuging damaged the eelworms.

After allowing the suspensions to settle for about 1½ hrs, most of

the water was withdrawn with a fine pipette, and 1 ml of a known antibiotic or a filtered broth containing antibiotic was added to the larvae in each dish. The covered dishes were kept for 24 hrs at 24° C. Nutrient broths in which streptomyces or bacterial isolates had grown were used as a source of antibiotics. The broth was used after the fungus had been allowed to grow long enough to enable the titre of the antibiotic, when assayed against *Staphylococcus aureus,* to reach a maximum. Before filtering, the pH value of the broth was adjusted to 6.0 with 1 per cent phosphoric acid, to ensure absence from the broth of free ammonia, which is toxic to eelworms.

Effect on powers of penetration of the eelworm

At the end of 24 hours as much broth as possible was drawn off with a fine pipette, and the eelworms were transferred to a graduated centrifuge tube, made up to 2 ml and then centrifuged at 347 g (1500 r.p.m.) for 2 minutes. The supernatant liquid was then drawn off, leaving 0.5 ml in each tube, which was made up to 2 ml and again centrifuged. This process was repeated three times, when the rootknot larvae were considered free of the broth. (Note: the stem eelworm, *Ditylenchus dipsaci* (Kühn) Filipjev, is also successfully spun down in the same manner, except that the centrifuge speed can safely be reduced to 154 g (1,000 r.p.m.) for one minute).

The eelworms were transferred from the centrifuge tube and placed on the surface of vermiculite around a tomato seedling, already established in a tube, with primary roots about 4 cm long. The tubes were surrounded by dark paper and placed in racks inclined at an angle of approximately 45°. Each tube was marked so that it would always be in the rack in the same position, i.e. the side with the roots underneath. The tubes were kept in the glasshouse, maintained at a temperature of 21-24° C during the day and at about 2° C lower at night. During the winter months the daylight was supplemented by artificial lighting, so as to give a minimum photoperiod of sixteen hours.

After twelve days the roots of two of the four replicates in each treatment were stained in cotton blue lactophenol and examined for penetration.

The reproductive test

The other two replicates in each treatment were allowed a further period of growth. On appearance of egg masses, approximately nine weeks after inoculation, two tomato seedlings were placed alongside

the original plant (two in case one died). Ten days later these seedlings were examined for the presence of second generation eelworms that might have emerged from egg sacs on the original plant. Where the eelworms had emerged in large numbers, their presence was easily observed from the swollen root tips of the young seedlings and it was quicker to inspect the young tomato seedlings rather than the original plant. When the root tips were not obviously swollen the seedlings and the original plant were stained in cotton blue lactophenol and examined for the presence of larvae.

RESULTS

Table I describes the composition of the media used for the production of a submerged culture fermentation of the isolates.

Table I

Composition of media used for fermentation

Soya bean	= S.	Soya bean meal 3 g, distillers' solubles 0.75 g, sodium chloride 0.25 g, distilled water to 100 ml; pH adjusted to 7.6 ± 0.1.
Beet molasses	= B.	Beet molasses 2 g, C.S.L. 1 g, soya bean meal 1 g, oatmeal 1 g, chalk 0.5 g, ferric citrate 0.5 ml, distilled water to 100 ml; pH adjusted to 7.4 ± 0.1.
Oatmeal	= O.	Oatmeal 2.5 g, calcium carbonate 2.0 g, dextrose 0.5 g, distilled water to 100 ml; pH adjusted to 6.8 ± 0.1.
K.L.M.	= K.	Composite mixture 1.75 g, dextrose 0.25 g, maltose 0.126 g, brown sugar 0.126 g, malt extract 0.126 g, yeast extract 0.03 g, distilled water to 100 ml; pH adjusted to 7.3 ± 0.1.

Table II gives the results from eleven routine tests. Of four replicate tomato plants grown in tubes the roots of 'a' and 'b' were stained for larval counts after the number of days shown in the second column, 'c' and 'd' were grown on to test for the development of egg sacs and the production of second generation larvae.

In tests A and B the broths H727, H997, H916, H939 and J45 were found to contain ammonia and no eelworms invaded the roots; the pH value had not been adjusted. Broths H997 and J3 in tests C and D were each adjusted to three different pH levels. At values of 7 and over no eelworms invaded the roots owing to the presence of ammonia. Some of the ammonia is almost certainly present as ammonium carbonate or bicarbonate, therefore even at pH values approximating 7 there will be some ammonia, since at this pH free ammonia is available owing to its equilibrium with ammonium and hydroxyl ions.

TABLE II

Counts of eelworms obtained after various treatments

Test	Days after inoculation with eelworms	Antibiotic broth number	Medium	pH	Approximate number of eelworms present in tomato roots		Approximate number of egg sacs produced	
					a	*b*	*c*	*d*
A	8	H727	S	9.0	0	0	0	0
		H997	S	9.0	0	0	0	1
		Water		7.2	4	14	22(2)	D
B	8	H793	O	7.4	36	73	++(2)	++(2)
		H900	O	7.3	41	57	+(2)	+(2)
		H916	B		0	0	0	0
		H939	S		0	0	0	0
		J45	B	8.0	0	0	0	0
		Water		7.2	21	16	+	+
C	12	H997	S	8.4	0	0	0	0
		H997	S	7.0	0	0	0	0
		H997	S	6.0	37	81	+	+(2)
		Water		7.2	62	41	+	+
D	9	J.3	B	8.5	0	0	0	0
		J.3	B	7.0	0	0	0	0
		J.3	B	6.0	16	2	+	+
		Water		7.2	17	36	D	D
E	12	J.1	B	6.2	22	19	+(2)	+(2)
		J.119	O	7.4	22	57	41(2)	+(2)
		J.150	O	7.1	23	24	33(2)	11(2)
		J.159	B	6.8	12	4	22(2)	+(2)
		J.161	O	6.8	13	20	10(2)	19(2)
		Water		7.2	52	7	3	+(2)
F	12	J.10	S	6.9	14	24	22(2)	20(2)
		J.106	B	7.5	14	36	26(2)	28(2)
		J.109	S	6.8	34	26	31(2)	21(2)
		J.118	B	7.4	3	15	10(2)	11(2)
		J.130	O	7.1	6	41	35(2)	22(2)
		Water		7.2	36	38	11(2)	27
G	13	H773	O	7.2	23	2	33(2)	20
		H923	B	7.5	0	9	10(2)	15(2)
		Water		7.2	36	39	1	26
H	13	Cycloheximide 200 µg/ml		5.8	0	0	0	0
		H854	O	6.8	6	16	50(2)	70(2)
		H935	O	7.5	36	30	D	35(2)
		Water		7.2	36	58	12(2)	+(2)

(*continued on the next page*)

TABLE II (*continued*)

Test	Days after inoculation with eelworms	Antibiotic broth number	Medium	pH	Approximate number of eelworms present in tomato roots		Approximate number of egg sacs produced	
					a	*b*	*c*	*d*
J	13	H663	K	6.3	26	47	++(2)	++(2)
		J144	B	6.0	38	46	9(2)	+(2)
		Water		7.2	77	54	++(2)	+(2)
K	13	J113	S	6.9	0	0	1(2)	1
		J114	S	6.7	17	0	26(2)	19
		Water		7.2	70	49	++(2)	+(2)
L	15	J179	B	5.8	19	63	6(2)	35
		J266	O	5.8	20	86	18(2)	40
		1) Undecylenic acid 2%		6.4	0	0	0	0
		Water		7.2	26	118	30(2)	46(2)

1) TARJAN & CHEO recently reported that undecylenic acid is phytotoxic to plants.

Key: + = 0-30 sacs ++ = more than 30 sacs D = plant dead (2) = second generation eelworms

Medium: S = soya bean O = oatmeal K = K.L.M. B = beet molasses

After treatment with broths H793 and H900 (test B) and all broths in test E, F, G, H, J, K and L, eelworms were still found in the roots showing that the broths at these concentrations contained no antibiotic lethal to root-knot eelworm. A 2% solution of undecylenic acid (test L) was lethal, no eelworms being detected in the roots of the test plants. However, it has recently been reported by TARJAN & CHEO (1956) that undecylenic acid is phytotoxic.

DISCUSSION

The technique for testing the nematicidal value of antibiotics failed to show any that were lethal to root-knot nematodes. Wherever the pH of the broth was unduly high, ammonia was liable to be present and had a lethal effect on the eelworms. The technique indicated this clearly which suggests that lethal effects from the antibiotics would have been shown had they existed. The lethal effect of undecylenic

acid was also clearly shown. Throughout the two halves of the test (concerned with invasion and development respectively) are in agreement.

ACKNOWLEDGMENTS

I wish to thank Professor B. G. Peters for his advice, Dr. J. W. Seinhorst for suggestions; and Miss P. Beard for technical assistance.

ZUSAMMENFASSUNG

Eine Methode für die Bewertung der Aktivität von Antibiotika gegen *Meloidogyne incognita* var. *acrita* wird beschrieben. Die Nematoden wurden nach der Behandlung zur Entfernung des Antibiotikums gewaschen und auf die Wurzeln von Tomatenpflanzen, die in 'Vermiculite' in Glasröhren angezogen worden waren, übertragen. Die Wurzeln wurden periodisch untersucht, um zunächst zu ermitteln, ob die Nematoden in die Wurzeln eingedrungen sind und später, ob sich die Nematoden in den Wurzeln normal vermehrt haben. Fleischbrühen mit hohen pH-Werten enthielten Ammoniak, das die Nematoden tötete. Undecylen-Säure war ebenfalls tödlich. Fleischbrühe an sich war unwirksam gegen die Nematoden.

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Received for publication: December 4, 1957

Nematologica III (1958): 149-153. E. J. Brill, Leiden

THE CONCENTRATION OF POTATO ROOT DIFFUSATE UNDER REDUCED PRESSURE

BY

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Potato root diffusates may need to be concentrated for two reasons; to reduce to manageable quantities the large amounts of leachings collected during the growing season and to determine whether there is an optimum concentration for the active factor in the leachings.

Preliminary experiments in this connection have been described by HAGUE (1954). FENWICK (1957) has shown that the temperature at which diffusates are concentrated has very little effect on their activities so long as 40° C is not exceeded during the concentration. The present paper describes the concentration of samples of diffusate obtained by two methods:

1. by leaching potatoes growing in soil with tap water,
2. by leaching potatoes growing in a clear silica sand (Loch Aline) with distilled water.

Samples of diffusate collected by the first method will be referred to as 'ordinary' diffusates and those collected by the second method as 'pure' root diffusates.

EXPERIMENTAL PROCEDURE AND RESULTS

The concentrations were carried out either at 5° C or at 30° C. At 5° C water was removed at a pressure of about 0.5 mm mercury by a vacuum pump. The water distillate was collected in a trap immersed in a freezing mixture (solid carbon dioxide in alcohol) at about -70° C so as to remove the water as ice; a second small trap was provided to protect the pump from contamination by water. The apparatus was capable of removing water at about 800 ml per hour under optimum working conditions. Both ordinary and pure diffusates were concentrated by this method.

A metal water pump was used to concentrate diffusates at 30° C, the pressure being about 5 mm of mercury; water was removed at 400 ml per hour.

Pure diffusates were reduced to the solid state. The volume of the leachings, having first been concentrated to about 75 ml by the method outlined above, was then lowered to a temperature of about -70° C and the final evaporation was carried out under reduced pressure in a desiccator, the excess water being absorbed in phosphorus pentoxide. The ratio of the original volume of the leachings to the volume of the concentrate is known as the concentration factor. For bioassay, the concentrated fractions were diluted on a logarithmic scale (see FENWICK 1952).

The cysts used for the bioassay of concentrated fractions of the diffusates were obtained either from field populations or from populations raised in pots (cysts of known age). The experimental techniques used in hatching larvae from root diffusate, and in estimating the number of larvae which emerged, were essentially those described by FENWICK (1949). The number of larvae which emerged in 3 weeks at each dilution of the diffusate was estimated as a proportion of the total viable contents of the cysts, the proportional response being plotted against the logarithm of the dilution of the root diffusate.

In a preliminary experiment leachings of an ordinary root diffusate were concentrated at 5° C by a factor of 40 : 1. The results of the assay (Table I) show that there is an optimum emergence at a dilution of

TABLE I

The bioassay of concentrated fractions of ordinary leachings of potato root diffusate distilled at 5° C

Dilution of the concentrated diffusate	Emergence larvae/cyst	Percentage emergence
1/1	119	17.0
1/4	199	28.5
1/16	240	34.3
1/64	229	32.8
1/256	111	15.9
1/1024	80	11.4
1/4096	46	6.6
Original diffusate (equivalent to a dilution of 1/40)	264	37.8

about 1/16 of this concentration. It is calculated that in the various processes, the root diffusate had lost about 3 % of its activity. A similar result was obtained when pure leachings were concentrated at 5° C (Fig. 1). During this concentration, losses of activity up to 12 per cent

were demonstrated in assays using all three populations of cysts. However the experiment did show that leachings could reach a concentra-

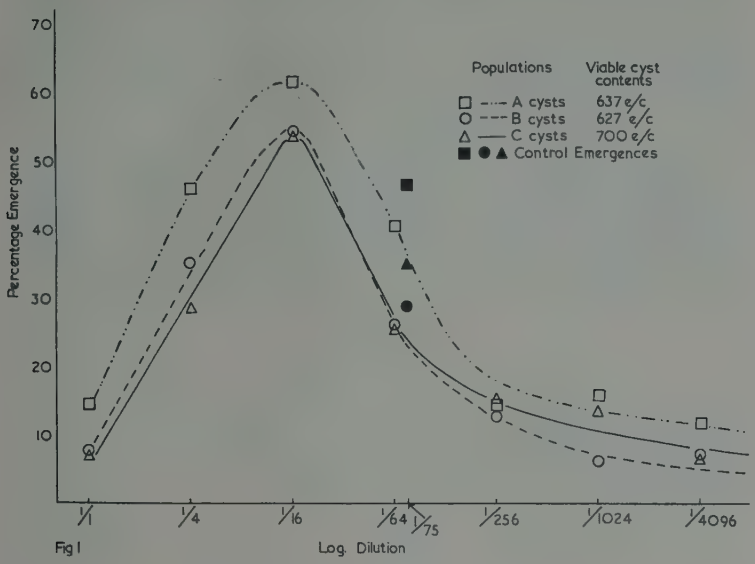

Fig. 1. The concentration-response curves obtained when pure leachings were concentrated at 5° C.

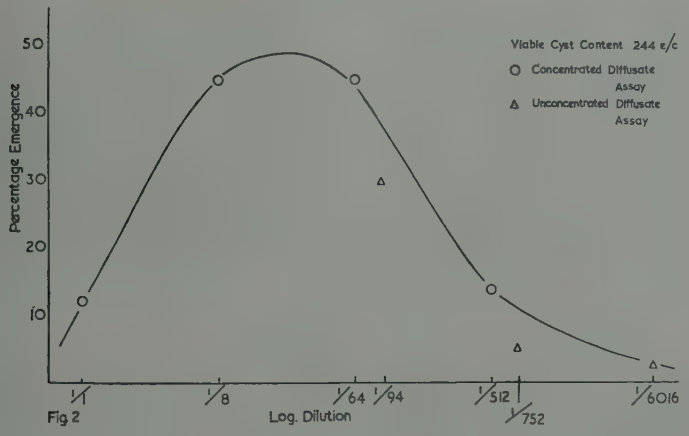

Fig. 2. The concentration-response curves obtained when pure leachings were reduced to the solid state.

tion at which their activity was greater than that of the originally collected diffusate.

2350 ml of pure leachings were reduced to the dry state giving 105 mg of solid material. Redissolving the solid in 25 ml of distilled water gave a concentration factor of 94 : 1. The results of the assay (Fig. 2) show that there is a marked optimum concentration and that no loss of activity could be detected.

Leachings of ordinary diffusates were concentrated 6-fold at 30° C as described above and the results of an assay of one such concentrate are shown in Table II. No loss of activity occurred and the activity of the diffusate in the original leachings appeared to be close to the optimum demonstrated in the assay of the concentrated fractions.

TABLE II

The bioassay of leachings of 'ordinary' root diffusate distilled at 30° C

	Concentrate:		Original leachings:	
Dilution of the diffusate	Emergence larvae/cyst	Percentage Emergence	Emergence larvae/cyst	Percentage emergence
1/1	288	45.2		
1/6	562	88.2	500	78.5
1/36	288	45.2	200	31.4
1/216	116	18.2	125	19.6
1/1296	25	3.9	63	9.9

DISCUSSION

The experiments described above show that the concentration-response relationship takes the form of a hump-shaped curve, a result similar to that reported by WINSLOW and LUDWIG (1957) for beet leachings. Thus two widely spaced concentrations of the stimulant may produce similar responses, a fact which introduces complications into the bioassay of the active factor, for which no chemical assay is as yet available. As in the case of beet leachings, the inhibitory effects observed at high concentrations of ordinary root diffusates might be attributed to the presence of soluble soil nutrients which are then at concentrations near saturation. However a hump-shaped relationship has also been observed for anhydrotetronic acid (BISHOP, 1955), and for the pure diffusates in the experiments reported here, which considerations suggest that the hatching factor itself may show a similar concentration-response relationship. Until a chemical assay is found no clear alternative is available. For storing leachings of ordinary diffusate a concentration factor of about 10 : 1 would appear suitable

and the leachings could be distilled without loss of activity at 30° C which is in agreement with FENWICK's finding as noted above.

It has been shown that the concentration of the original diffusate leachings was close to its optimum and thus it would be satisfactory to use the original leachings to ascertain the emergence of larvae from cysts treated chemically. Reducing the volume of leachings is necessary only when the problem of storage becomes acute.

The methods outlined above may be of use in reducing to manageable volumes the large bulks of leachings which have been collected by various workers for the chemical characterization of the hatching factor. Until the identity of the stimulating agent has been discovered, its absolute activity cannot be evaluated in terms of standard solutions of a known active substance.

ZUSAMMENFASSUNG

Methoden werden beschrieben, mit deren Hilfe aktive Kartoffelwurzeldiffusate konzentriert werden können. Die Reaktionen der ausschlüpfenden Nematodenlarven auf verschiedene Konzentrationen ergeben, graphisch ausgedrückt, eine konvexe Kurve. Die Aktivität der Diffusate war nicht beeinträchtigt, wenn die Destillationen bei 5° C und bei 30° C ausgeführt wurden. Die Wirksamkeit der Wurzeldiffusate ist von der gleichen Grössenordnung wie die optimale Aktivität der konzentrierten Fraktionen.

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Received for publication: December 23, 1957

Nematologica III (1958): 154-167. E. J. Brill, Leiden

THE POTATO EELWORM HATCHING FACTOR 7. FURTHER METHODS FOR CONCENTRATION OF THE FACTOR

BY

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The first extensive studies on the chemical nature of the potato-root eelworm (*Heterodera rostochiensis* Woll.) hatching factor were carried out by Todd and his associates (CALAM, RAISTRICK & TODD, 1949; CALAM, TODD & WARING, 1949; MARRIAN, RUSSELL, TODD & WARING, 1949) who investigated methods of purification of the active factor and determined some of its characteristics. The crude starting material used in their work was obtained by growing tomatoes in pots or trays of soil and leaching the soil with water at frequent intervals. The hatching factor contained in these leachings was concentrated by adsorption on charcoal and was eluted with aqueous acetone. Concentration of this eluate at a low temperature *in vacuo* yielded a light brown water-soluble powder which was stated to contain 60-70 % of the active material in the leachings.

Further purification of the hatching factor was achieved by dissolving this powder in dilute sulphuric acid and subjecting the solution to continuous ether extraction which removed most of the active material. Since it appeared that the factor was acidic in nature, attempts were made to obtain further purification by preparation of metal or alkaloid salts and by chromatography on silica gel, but these were, on the whole, unsatisfactory. The alkaloid salts were active but attempts to fractionate them or to recover the free acid resulted in heavy losses and in many cases in complete inactivation.

The experiments now reported were a continuation of the above studies and were carried out with the advantages of a greatly increased supply of starting material and a much improved bioassay method.

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1) Merck Fellow in the Natural Science of the National Research Council of Canada.

EXPERIMENTAL

Bioassay of the hatching factor

All of the bioassays reported in this paper were carried out by Dr. D. W. Fenwick and his associates at Rothamsted Experimental Station. The methods have been described in detail (FENWICK, 1940; 1949; 1952) [1]) and only a brief summary will be given here.

In principle, the bioassay was the same as that used in the earlier chemical studies. Given numbers of eelworm cysts were incubated in test solutions for a definite period of time and the number of larvae emerging was taken as a measure of the activity of the solutions. However, the number of cysts used in each individual test was increased to at least 100 and in most assays to 200-400. Replication was from three to tenfold according to circumstances and the time of incubation was increased from 2-3 days to 14 days with a preliminary count at 7 days.

Bioassay of an unknown solution at a single dilution can give only a qualitative measure of its hatching activity since the hatch is proportional to the concentration of hatching factor over only a small range of concentrations and tends to be inhibited at higher concentrations. (CALAM, RAISTRICK & TODD, 1949; HAGUE, 1954). Therefore, in this study, samples were usually assayed at a number of different dilutions and an attempt was made to have at least two points on the proportional part of the hatching curve. On this part of the curve, the total hatch is roughly proportional to the logarithm of the concentration of the hatching factor and activities have been expressed in terms of L.A. (log activity) values (FENWICK, 1952). An L.A. of 0 is taken as that concentration of hatching factor which just fails to give a hatch significantly higher than water. An L.A. of 1 therefore represents 10 times this limiting concentration, L.A. of 2 represents 100 times, etc.

The number of larvae emerging in any given concentration of hatching factor varies with the population of cysts used since these may differ greatly in the number and hatchability of eggs which they contain. It is generally easier to get high hatches in summer than in winter and other factors such as the degree of maturity and previous history of the cysts are involved. Therefore it is imperative for quantitative assays that each set of unknowns be compared at a series of

1) The bioassay technique is described in full by FENWICK in an article which will appear shortly in the Journal of Helminthology. An article on attendant errors is in course of preparation and probably appears in the same journal.

dilutions with a control. For those studies, a quantity of crude leachings was set aside at low temperature and was retested with each new assay.

Supply of starting material

Nearly all of the material used in these studies was produced at the Department of Horticulture, University of Reading under the supervision of Professor R. H. Stoughton. The plants were grown under glass in beds of sand 2-4 feet in width and 25-100 feet in length. The 12 beds comprised a total of 1500 square feet. Each bed was equipped with a central drain which emptied into a tank for collecting the leachings. Later the growing space was approximately doubled by the construction of additional outdoor frames.

Both potatoes and tomatoes were grown, the usual procedure being to plant potatoes early in February and replace them with tomato seedlings when the activity of the potato leachings began to decrease markedly. The potatoes normally began to produce highly active leachings during the first or second week of March and the activity was maintained for a period of about six weeks. Similar results were obtained with the tomatoes but the induction period was somewhat shorter. Although leaching was carried out once a week, later experiments indicated that this could probably be done 2 or even 3 times weekly without decreasing the potency of the leachings or having a harmful effect on the plants. A total of 650 gallons was collected from the 12 beds under glass at each leaching. The plants were supplied with a nutrient medium, the composition of which is given in Table I, and this was normally replaced at the end of each leaching.

TABLE I

Composition of nutrient medium

	g/litre
$Ca(NO_3)_2.4H_2O$	1.69
Na_2HPO_4	0.194
K_2SO_4	0.335
$MgSO_4.7H_2O$	0.505
H_3BO_3	0.00545
$MnSO_4.4H_2O$	0.00396

The most active leachings had L.A. values ranging from 2.5 to 3.5 and it is probably not profitable to use leachings with an L.A. of less than 2.0, as a difference of one unit in L.A. value represents a 10-fold difference in the concentration of the active compound.

A few preliminary experiments were carried out on leachings supplied by Dr. Fenwick from potatoes grown in pots of soil. These had an L.A. value of 2.5-3.0 but were deeper yellow in colour and extracts from them usually appeared less clear than those from leachings of plants grown in sand.

In the early stages of this work, a number of plant extracts were examined as possible alternative sources of active material. Aqueous extracts of 10 different species of Solanaceae were prepared by grinding them with 2-2.5 volumes of water in a Waring blendor, allowing to stand overnight and then separating any solid material. The filtrates had little or no hatching activity, with the exception of potato and tomato in which the activity approached that of the root diffusates. However, the total solids in these filtrates was about 100 times the amount in leachings from the beds and the filtrates were therefore not considered to be useful alternative sources of hatching factor. Aqueous extracts of the roots of the tomato plant were slightly less active than those of the stems and leaves, and extracts of tomato fruit (either green or ripe) and of potato tubers contained only traces of activity. Attempts to use other solvents such as alcohol or acetone, rather than water, for extraction appeared to offer no advantages.

Concentration of the hatching factor

As indicated in the preceding section, several thousand gallons of leachings were collected during a season and the first problem in purifying the hatching factor was to reduce the volumes to manageable size. In all of the earlier work (Calam, Raistrick & Todd, 1949), and for most of the studies covered by this report, it was accomplished by adsorbing the factor on charcoal and eluting it with acetone-water (70 : 30). Although Calam et al. (1949) claimed to have recovered 60-70 % of the active material by this method, accurate bioassays with the improved method indicated that under the conditions of the present experiments the recovery rate was of the order of 10 % or less. Other tests showed that the amount of charcoal used (1 lb. per 100 gallons) removed nearly all of the activity from the leachings. It was also found that using larger volumes of aqueous acetone to elute the charcoal did not increase the yield of active material appreciably, although elution by percolating aqueous acetone through a column of the charcoal was more efficient than the batch method used earlier. It therefore appears that a large proportion of the hatching factor was adsorbed strongly on the charcoal, although further quantities of active

material could be obtained by subsequent extraction with phenol.

Another objection to the use of charcoal is indicated by the isolation of inorganic material comparable in weight (75 mg/100 g. charcoal) and appearance from charcoal which had been suspended in either tap water or root leachings.

In view of these objections, consideration was given to other methods for concentrating the hatching factor. A promising alternative was simply to concentrate the leachings *in vacuo* at temperatures below 45°. It was shown that this could be accomplished without loss of activity provided the pH was maintained between 5 and 7. When either crude leachings or charcoal eluates were concentrated, their pH tended to rise and dilute acid was normally added to keep the pH below 7. About 1 ml of 3N sulphuric acid was required for each litre of leachings.

Large scale concentration of leachings was accomplished in vacuum concentrators made available through the kind co-operation of Professor Crossley of the Dairy Department of the University of Reading. With these, 80 gallons of leachings were concentrated to one gallon in 2-3 working days. At this point the concentrated leachings contained about 5 % total solids and further concentration tended to cause marked precipitation and loss of activity.

In an alternative procedure, devised by Dr. J. B. Armitage, it was established that the active factor, which contains an acidic grouping, can be absorbed by a strongly basic ion exchange resin and subsequently can be displaced by mineral acid. In the procedure adopted root diffusate was percolated through a column of the cation-exchange resin, Zeokarb 225 and the eluate brought onto a column of Deacidite FF, an anion-exchange resin, when all the cations were retained. 3N-Hydrochloric acid (1 bed volume) eluted the absorbed acids and the eluate had activity comparable with the original root diffusate. Portable "Permutit" units of Zeokarb 225 and Deacidite FF were able to concentrate several hundred gallons of the crude root diffusate each week. Preliminary experiments have indicated that if the strongly acidic eluate from the Deacidite FF column is treated with the weak base Deacidite E, the mineral acids are absorbed but not the weaker organic acids. This method of purification is still under investigation.

Elimination of inorganic salts present in the concentrates

When leachings were concentrated by the charcoal adsorption method, the charcoal eluates contained 75 % or more of inorganic salts and it is

probable that the inorganic fraction was even higher in the vacuum concentrates. In the latter case, much of this inorganic material appeared to come from the tap water used for leaching and additional amounts were derived from the nutrient solution added to the beds. (Table II).

TABLE II

Solids contents of leachings from potato beds

	mg/100 ml
1. Beds given full nutrient and leached with tap water	70
2. Beds given no nutrient and leached with tap water	50
3. Solids content of tap water	36

In earlier work (CALAM, TODD & WARING, 1949) the active factor was separated from the inorganic salts by acidifying an aqueous solution of the charcoal eluates and extracting continuously with ether, whereupon the active substance was found in the ethereal extract. It has not been found possible to repeat this result in the present study (cf. MASSEY & NEAL, 1953), but three alternative methods have been found. The active material may be extracted from an acidified aqueous solution using either *n*-butanol or *sec*-butanol, and it may be extracted from a neutral aqueous solution by phenol. In each case the bulk of the inorganic material remains in the aqueous phase. Alternatively, the active material may be adsorbed on a column of powdered cellulose, and the inactive inorganic salts and organic contaminants eluted with ethyl acetate containing increasing proportions of ethanol. The active material may then be eluted with 50 % aqueous alcohol. It was noted that the R_F values for the active fractions varied with the age of the plants and it is likely therefore that more than one active principle is involved in the hatching process. However, the chromatographic method of separation was lengthy and phenol extraction has generally been preferred because it gave at least as good recovery as the butanol extractions and extracted much less inorganic material. From equal volumes of vacuum concentrate, the *n*-butanol extract weighed 540 mg, the *sec*-butanol extract 900 mg and the phenol extract 245 mg. The phenol recoveries were consistently high, whereas at times the hatching factor appeared to be partially inactivated during the butanol extractions.

The procedure used in the phenol extraction was as follows: 600 ml of vacuum-concentrated leachings (pH 6), equivalent to approximately 10 gallons of leachings were extracted with 3 × 200 ml of water-saturated phenol. The combined phenol extract was then mixed with

1800 ml of ether and back-extracted with 200 ml and then 4 × 100 ml of water, and the combined aqueous extract was washed with 3 × 200 ml of ether to free it from phenol. The aqueous extract was then concentrated to small bulk in vacuo. It normally contained about 500 mg of total solids, or about 1 % of the total solids in the vacuum concentrate. The yield of phenol extract was thus about 5 g/100 gal. of leachings while the yield from the charcoal elution method was only 0.1 g/100 gal. and activity tests indicated that the extract from the vacuum concentrate was at least as active per unit weight as that obtained by charcoal elution.

Further purification of the hatching factor

Paper chromatography

Throughout this work it has been necessary to rely on the biological assay to detect the presence or absence of the hatching factor. Since the assay is extremely laborious and time-consuming it is obvious that any chemical means of detecting the active compound would be invaluable.

A number of experiments have indicated that the hatching factor is an acidic compound and it should therefore be possible to detect it on paper chromatograms sprayed with indicator. With this in mind, the phenol extracts and many of the fractions derived from them have been chromatographed on paper in an attempt to identify some acidic compound with a characteristic R_F value in the active fractions. The solvent system used most frequently was the upper phase from a mixture of *n*-butanol (100 ml), water (100 ml) and formic acid (28.75 ml) (Lugg & Overell, 1948). The chromatograms were run downwards, and at the end of the run the papers were allowed to dry for an hour at room temperature and then sprayed with a solution of bromophenol blue in alcohol (40 mg/100 ml), which had been neutralized with alkali to a reddish-blue tinge. Some acid spots were immediately apparent on the papers while others appeared gradually as the residual formic acid evaporated from the paper and the background became deeper blue.

Mono-basic acids gave well-defined spots when solutions containing 100 mg/10 ml were applied to the paper. Unknown mixtures were usually applied at 10 times this concentration. The phenol extracts derived from charcoal eluates gave yellow acid streaks in this chromatographic system, extending from R_F 0.15 and with most of the colour concentrated between R_F 0.60 and 0.85. The cations in the extract gave

a deep blue spot near the origin and when they were removed, further acid spots were revealed. Some attempts were made to elute the hatching factor from these chromatograms but no active material could be recovered. Another chromatographic solvent which has been used for the analysis of active fractions is ethanol-15N ammonium hydroxide-water (20 : 1 : 4). Acidic and basic zones were located by spraying the paper with B.D.H. Universal Indicator at pH 8. Attempts to replace paper strip chromatography by columns of washed powdered cellulose and eluting with various neutral solvents failed to achieve the separation of any single active substance.

The paper chromatograms did, however reveal that the phenol extracts contained a variety of acidic compounds and several different techniques have been used in attempts to separate this mixture and isolate the pure hatching factor.

Counter-current distribution

Preliminary experiments on the distribution of activity between water and butanol showed that at pH 1 the active substance was mainly in the butanol layer, but at pH 6.5, where the salt form might be expected to predominate, the active substance was mainly in the aqueous layer. A few attempts were therefore made to separate the material by counter-current using as a solvent pair either *n*-butanol or *sec*-butanol and phosphate buffers of pH 5.9 to 6.4 (Craig, Golumbic, Mighton & Titus, 1945; Sato, Barry & Craig, 1947), but the results were discouraging. The bioassay did not lend itself to testing large numbers of fractions and in small series of transfers there was no evidence that the hatching factor was being freed from other impurities. Added to this was the difficulty of recovering active material free from the phosphate buffer, and there was also evidence that the factor was being partially inactivated. This method was therefore abandoned.

Silica gel chromatography

Partition chromatography on silica gel was also examined, as a possible method of purifying the hatching factor. The silica gel was prepared according to the directions of Isherwood (1946) and 4.5 g were used in a column 1.2 cm diameter. Dilute sulphuric acid (0.5N) was used as the stationary phase and the column was eluted with 100 ml each of 15% butanol in chloroform, 35% butanol in chloroform and finally 50 % butanol in chloroform. Fractions of 10 ml were collected

and titrated with 0.02N potassium hydroxide in ethanol, using phenolphthalein as indicator (Bulen, Varner & Burrell, 1952). Phenol extracts (50 mg) of charcoal eluates from both tomato and potato leachings were chromatographed on these columns. Three distinct acid peaks were obtained with the tomato extract and four with the potato (Fig. 1). The positions of these acid peaks were related to their R_F values in the butanol-water-formic acid system on paper, acids having higher R_F values being eluted first from the column. This relationship is to be expected since both techniques depend basically on partition of the acids between dilute aqueous acid and butanol.

Unfortunately, very little hatching activity was found in the various

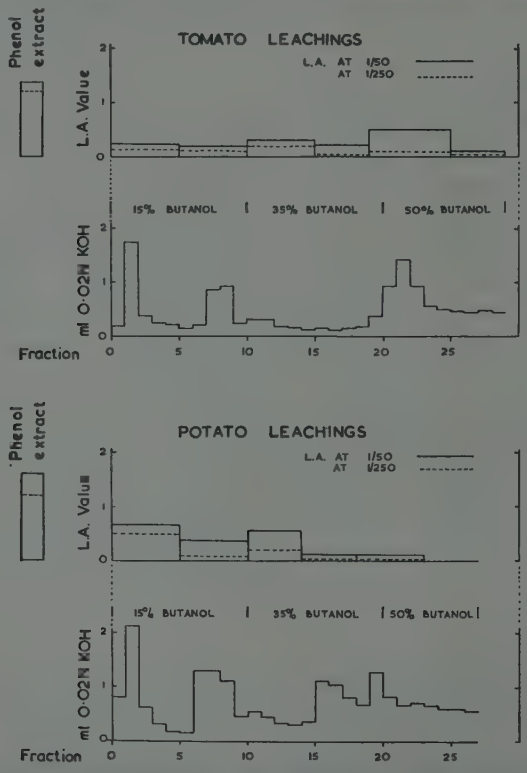

Fig. 1. Silica Gel Chromatography. The distribution of hatching activity (L.A. value) and acid peaks among successive fractions eluted from the column when phenol extracts of charcoal eluates from tomato and potato root leachings were chromatographed on silica gel.

fractions, possibly because the hatching factor was not eluted from the column or because it was inactivated under the conditions of the experiment. The reasons for the lack of success with these columns were not investigated extensively because of the promising results obtained with ion exchange resins.

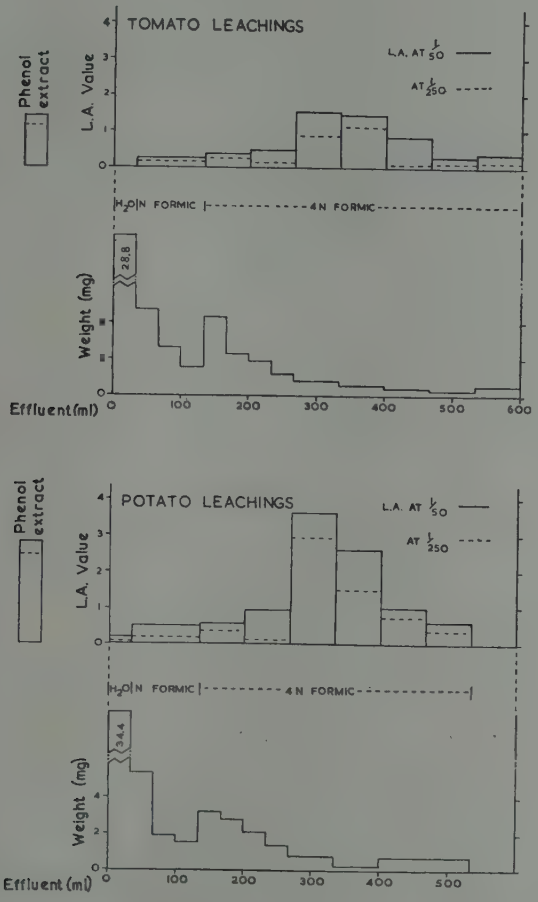

Fig. 2. Ion Exchange Chromatography. The distribution of hatching activity (L.A. value) and total solids among successive fractions eluted from the column when phenol extracts of charcoal eluates from tomato and potato root leachings were chromatographed on Dowex 2-x10 (200-400 mesh) in the formate form.

Ion exchange chromatography

The experiments in the use of this technique were based on the work of Busch, Hurlbert & Potter (1952). Dowex 2-×10 (200-400 mesh) in the formate form was used in most experiments. Aliquots of 50 mg of phenol extracts were dissolved in a small volume of water and added to columns of 1 cm × 11.5 cm. The columns were eluted first with 33 ml of water to remove cations and this was followed by 100 ml of 1N formic acid and then larger volumes of 4N formic acid. The eluted fractions were first concentrated by distillation in vacuo at a low temperature and were finally taken to dryness over phosphorus pentoxide and solid potassium hydroxide in a vacuum desiccator. They were redissolved in water for bioassay, each fraction (equivalent to 50 mg of phenol extract) being taken up in 10 ml. Results obtained with phenol extracts of charcoal eluates are shown in Fig. 2. Nearly all of the hatching activity was contained in the fractions between 250 and 400 ml of effluent. It appeared that most of the activity in the original phenol extract was recovered in these fractions but the total weight of active material was only 1-2 mg, or less than 5 % of the weight of phenol extract. This meant a yield of only about 5 mg of active material from 100 gallons of original leachings, and it was this result more than any other that led us to abandon the charcoal method of concentrating activity in favour of direct concentration in vacuo.

Fig. 3 shows some preliminary results obtained by chromatographing phenol extracts of vacuum concentrates on Dowex 2-×10. In these chromatograms, the active material seemed to be eluted earlier than that derived from the charcoal eluates, suggesting that the hatching factor may be altered by adsorption on charcoal.

The ion-exchange fractions from phenol extracts prepared by both methods were chromatographed on paper in the system described earlier. The active fractions contained acids of low R_F (< 0.30) and relatively high R_F (> 0.75) but had no characteristic spots to distinguish them from inactive fractions. Similar experiments were carried out using the resin Dowex 1-×2, a more weakly basic resin with a lower degree of cross-linking. Results obtained with phenol extracts of charcoal eluates from potato root leachings were essentially similar to those given in Fig. 2. Elution was effected with 2N rather than 4N formic acid but the distribution again indicated the presence of more than one active acid. When evidence was obtained for the presence of breakdown products from the Dowex 1-×2 resin itself in the eluate these experiments were discontinued.

It is known that a correlation exists between the R_F of compounds in butanol-water systems and their partition coefficients between butanol and water. (CARROLL, 1955). Acids with high R_F favour the butanol layer and those with low R_F favour the water layer. Earlier experiments had also shown that when crude or partially purified hatching factor was partitioned between water and butanol at an acid pH, most of the activity was found in the butanol layer. This suggests that the activity is associated with acids having a high R_F in the butanol-water chromatograms. Active fractions from the ion-exchange columns have

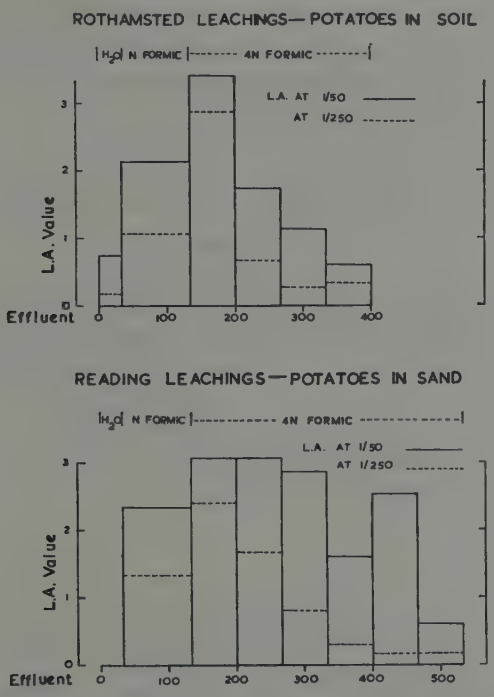

Fig. 3. Ion Exchange Chromatography. The distribution of hatching activity (L.A. value) among successive fractions eluted from a column when phenol extracts of vacuum concentrates from potato leachings were chromatographed on Dowex 2-x10 (200-400 mesh) in the formate form.

also been partitioned between butanol and water and preliminary activity tests on the two fractions were in agreement with this sugges-

tion. In another experiment, a fraction giving a single spot only with the butanol-formic acid-water system showed the presence of at least five acidic substances when chromatographed in the ethanol-ammonia-water system which demonstrates the need to use more than one solvent system in establishing the purity of a compound by this method. One of the active compounds in the ethanol-ammonia-water system had an R_F value of 0.40-0.45.

ACKNOWLEDGEMENTS

We are very grateful to Professor R. H. Stoughton, Department of Horticulture, University of Reading for the production of potato and tomato root diffusate over a period of five years; to Professor E. I. Crossley of the Department of Dairying, University of Reading for providing facilities for the large scale evaporation of the diffusate and to Dr. D. W. Fenwick and Mrs. E. Widdowson of Rothamsted Experimental Station for carrying out all of the biological testing. We also thank the Agricultural Research Council for its financial support of this project and Mr. G. G. Samuel for his interest in the work.

ZUSAMMENFASSUNG

1. Weitere Untersuchungen über den Kartoffelnematoden-Schlüpffaktor wurden mit einem stark vermehrten Ausgangsmaterial und mit einem erheblich verbesserten Biotest-Verfahren durchgeführt.

2. Sekrete aus den Wurzeln von Kartoffel- und Tomatenpflanzen, die in Sandbeeten angezogen worden waren, wurden im Vacuum bei Temperaturen unterhalb 45° C ohne bemerkenswertem Verlust der Schlüpfaktivität konzentriert. Diese Methode scheint der Adsorption des Aktivfaktors an Tierkohle und der Lösung in wässriger Aceton vorzuziehen zu sein.

3. Der schlüpffördernde Bestandteil wurde von der Hauptmasse des anorganischen Materials in dem konzentrierten Sekret durch Extraktion mit Phenol oder weniger wirksam mit Butanol getrennt. Das aktive Material wurde dann aus der Phenollösung durch Auflösen in Äther und Rückextraktion mit Wasser wiedergewonnen.

4. Phenolextrakte wurden weiter durch chromatographischen Ionenaustausch gereinigt. Darauf folgte die Fraktierung mittels Papierchromatographie. Mit einer Ausnahme gab es keine charakteristischen Säureflecke auf dem Papier, die die aktiven von den inaktiven Fraktionen unterschied. Die aktivsten Fraktionen, die aus der Ionenaustausch-Chromatographie erhalten wurden, enthielten wenigstens noch vier Säuresubstanzen. Das Problem der Trennung dieser schwachen Säuren ohne Zerstörung der biologischen Aktivität ist weiterer Untersuchung vorbehalten.

5. Versuche, den schlüpffördernden Bestandteil mit Hilfe einer aufsteigenden Arbeitsweise oder der chromatographischen Trennung an Silikat-Gel- oder Cellulosepulver-Säulen zu reinigen, waren erfolglos.

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Received for publication: January 6, 1958

BOOK REVIEW

C. L. DUDDINGTON: The friendly Fungi. A new approach to the eelworm problem. London, Faber and Faber, 1957. 188 pp., 24 figs. 21 sh.

After some introductionary remarks about plant parasitic nematodes and the possibilities of control, the author, who is a mycologist, comes to his theme. The interesting nematode-capturing fungi which live in the soil, are described at length and suggestions are made on the possibility of using these fungi for the biological control of soil-inhabiting eelworms. The investigations of LINFORD (1937-40) at Hawaii and DESCHIENS et al (1943-46) in France, concerning the control of *Meloidogyne* spp. with these fungi (*Arthrobotrys oligospora* Fres., *Dactylella* spp., *Dactylaria* spp., etc.) are reviewed. In both cases remarkable progress in destroying the nematodes by use of the fungi was made in the laboratory, however field experiments on a larger scale were not made.

In the last chapter a field experiment, done in England in 1954, is described extensively. Four treatments were given: (1) dung with predacious fungi, (2) dung only, (3)predacious fungi only, and (4) no treatment, on fields planted with oats and with potatoes, infested with *Heterodera major* O. Schmidt and *H. rostochiensis* Woll., respectively. Treatment (1) gave a better yield than the other treatments, so that it could be concluded that combination of dung with predacious fungi would be a promising method of control. However, on repeating the experiments in the following year (1955), no differences were found, and all yields were very poor because of the abnormal dry summer.

This is a very readable booklet which will interest all, concerned with nematode problems. It is well edited and richly illustrated. However, one objection must be raised. If thoroughly read, it becomes clear that facts which indicate that biological control of eelworms by these fungi is successful, are still very scarce. The photographs of the yields of potatoes on treated and untreated plots in particular, are likely to mislead layman to expect too much of control by these means when, in fact, results are not scientifically well founded.

P. A. VAN DER LAAN

Nematologica III (1958) : 169-172. E. J. Brill, Leiden

SPHAERONEMA MINUTISSIMUM N.SP. (SPHAERONEMATINAE: TYLENCHULIDAE)

BY

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Early in 1956, Mr. L. Boyce Thrower of the F.A.O. Technical Assistance Mission to Indonesia, sent some roots of *Citrus* sp. suspected of being attacked by nematodes. *Tylenchulus semipenetrans* was present, and careful examination, after staining in cotton blue lactophenol, also revealed the presence of minute spheres on the surface of some roots. At first sight these appeared to be fungal in nature, but on dissection from the root they were seen to be minute, spherical nematodes with the fore part imbedded in the root tissue and swollen in various ways. A number of these eelworms were dissected out, and from the soil which accompanied the roots, larvae of the same species were washed out. These larvae appeared to be newly hatched for they were similar to some found near to adult females on the roots. Eggs containing larvae about to hatch were also found and the size and structure of the larvae was comparable with those found free in the soil. The soil also yielded males and young females of a species of *Rotylenchulus* but no adult females of *Rotylenchulus* could be found on the citrus roots.

Although the number of specimens is limited and not as complete as one could wish, it seems worthwhile describing the nematode in the hope that other workers may find it and improve or emend the description from better and more abundant material.

All observations were made on material stained in cotton blue lactophenol and mounted in glycerine.

Sphaeronema minutissimum n.sp.

7 ♀♀ L = 0.112 mm. (s = ± 0.010 mm.) (measured range 0.101-0.129 mm.)
a = 1.7; b = ?; c = ?; V subterminal.
Holotype ♀ L = 0.101 mm; a = 1.5; b = ?; c = ?; V subterminal.

Female. The main part of the body is more or less spherical, about 66 μ in diameter with a relatively thick cuticle, up to 4 μ wide; together with the protruding neck and head the length of the eelworm is about 112 μ. The cuticle is quite smooth with no sign of any markings on it. Neither the excretory pore nor the anus were seen. The general appearance of the female is shown in Fig. 1a, which is a representation synthesised from several specimens. The lip region has a circum-oral elevation and, just behind this, the cuticle is thickened slightly and stains more deeply with cotton blue. This thickening is indicated in

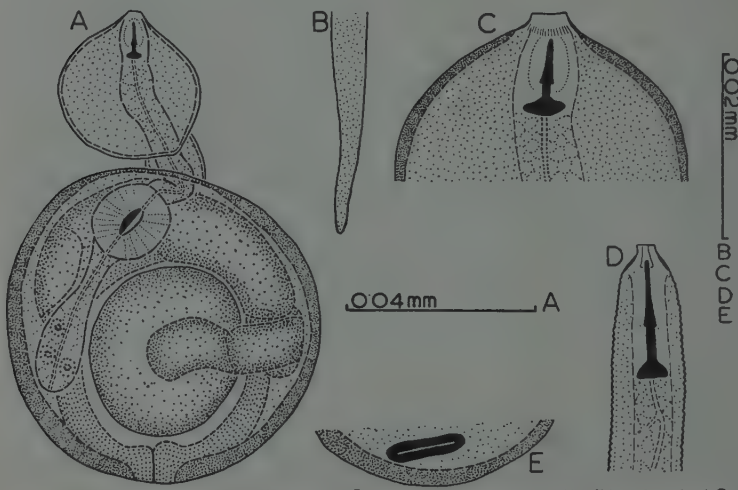

Fig. 1. *Sphaeronema minutissimum.* A ♀ eelworm; B larval tail; C head end of ♀; D head end of larva; E vulva in surface view.

Fig. 1c by a row of shading just below the circum-oral elevation. The stylet measures about 8 μ but is somewhat foreshortened (there was only a single female with a discernible stylet); its true length is probably 12-13 μ which is the same as the length of the larval stylet.

The fore part of the body is variously thickened depending presumably on its position between root cells. In some there is a single swelling joined to the body by a short neck as in Fig. 1a, in others there are two swellings. The oesophagus consists of a cylindrical precorpus, a well-defined, almost spherical corpus and a spathulate post-corpus joined to the corpus by a slightly narrowed isthmus. The corpus may be in the main body swelling as in Fig. 1a or may be found in a separate swelling of the fore part of the body. The nerve ring was not

discernible. The oesophageal lumen was well defined throughout its length but no opening of the dorsal oesophageal gland could be seen. The intestine could not be seen.

The vulva is almost terminal and the thick cuticle is interrupted where the vulval lips pass through it so as to be flush with the body surface (Fig. 1a & e). The vagina is very short and opens directly into the thick walled uterus containing a relatively large, almost spherical egg, about 37 by 32 μ in size. The rest of the ovary appears to consist of a few cells, the whole structure practically filling the body cavity and displacing the oesophagus to one side.

Larva (newly hatched)

29 larvae L = 0.315 mm. (s = ± 0.012 mm.) (measured range 0.296-0.338 mm.) a = 28.9; b = 3.6; c = ?

The slender body tapers fore and aft, and is annulated, except on the head, the annules being 0.7 μ apart. Neither the lateral line nor the anus were observed. The lip region is not offset and has the distinctive circum-oral elevation similar to that of the female. There is a slight thickening of the cuticle in the lip region which shows as a more deeply staining band. The stylet is 12-13 μ long, in three definite parts, a pointed fore part, a shaft slightly shorter than the fore part, and well-developed knobs, convex anteriorly, flat posteriorly. The opening of the dorsal gland is about 4.5 μ from the stylet base. The oesophagus occupied about a quarter of the body length but its structural details were obscure. The valve of the corpus was occasionally distinguishable at about half the oesophageal length and the excretory pore opened about a body-width behind the corpus. No nerve ring or hemizonid were discernible. The head and tail are depicted in Fig. 1d & b.

Type host. Roots of *Citrus* sp.

Type habitat. Citrus grove, Pasar Minggu, near Djakarta, Java, Indonesia.

Holotype. Female collected by L. B. Thrower, slide no. 80/2/1 in the collection of the Nematology Department, Rothamsted Experimental Station.

Relationships. Raski (1957) erected the family Tylenchulidae containing two subfamilies, Tylenchulinae Skarbilovich, 1947 with two genera, *Tylenchulus* Cobb, 1913 and *Trophotylenchulus* Raski, 1957, characterized by a posteriorly located excretory pore and a degenerate stylet in the male; and Sphaeronematinae Raski & Sher, 1952 also with two genera, *Sphaeronema* Raski & Sher, 1952 and *Trophonema* Raski,

1957, differentiated from the first subfamily by the normal position of the excretory pore and the absence of a stylet in the male.

The nematode described in this paper does not fall easily into any of the four genera above. It is similar to *Sphaeronema* in the shape and general organisation of the female and in the position of the excretory pore in the larva. Similarly it has affinities with *Trophotylenchulus* in the circum-oral elevation on the lip region of both female and larva. The constellation of characters shown by the new species might warrant the erection of a new genus, but for the present till more complete details of the nematode are forthcoming, it is considered that it should be placed in the genus *Sphaeronema* (Raski & Sher, 1952).

Differentiation. This new species differs from the only other in the genus, *Sphaeronema californicum* in its smaller size, in the possession of the circum-oral elevation on the lip region and in the almost spherical form of the egg.

ZUSAMMENFASSUNG

Sphaeronema minutissimum n.sp. wird beschrieben und abgebildet. Weibchen und Larven wurden an den Wurzeln von *Citrus* sp. gefunden. Der Körper des Weibchens ist fast sphärisch und etwas grösser als 66 μ im Durchmesser, mit einem vorspringendem vergrösserten Halsstück. Die Lippengegend hat eine circumorale Erhöhung und das Ei ist fast sphärisch.

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Received for publication: September 15, 1957

Nematologica III (1958) : 173-178. E. J. Brill, Leiden

OBSERVATIONS ON THE COLLECTION AND STORAGE OF POTATO ROOT DIFFUSATE

BY

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In a previous paper (WIDDOWSON 1958), some of the factors influencing the production of root diffusate by potted potatoes were discussed. Tests have now been made on methods of collecting diffusate, and observations made on the decline in hatching activity of diffusate stored under varying conditions.

Samples of the diffusates collected were assayed at one dilution usually $^1/_4$, on batches of 100 cysts in five fold replication, against a minimum of six dilutions of a stock diffusate. The cyst stocks used were all derived from the same source and contained approximately 100 eggs per cyst. The assay method is described in detail by FENWICK & WIDDOWSON (1958). The same paper recommends that for routine diffusate collection potatoes be grown in a mixture of three parts of sieved, unsterilized, loam, to one part of coarse horticultural sand, in $6^1/_2$ in. pots, (a volume of approximately 1000 ml. per plant). The plants should be leached thrice weekly for six weeks. Except where otherwise stated this is the method followed in these experiments; and tubers of approximately $2^1/_2$ oz. in weight, of the variety Arran Banner were used.

The question of the most suitable volume of leachate to take per $6^1/_2$ in. pot was investigated by leaching growing potatoes with large volumes of water. Five plants were selected from a set of fifty not previously leached, one week after breakthrough. Each pot was saturated and then slowly watered with 650 ml. of water and the diffusate was collected in successive fractions of 0-20, 20-40, 40-80, 80-160, 160-320, 320-640 ml. The last two fractions were pooled for the assay which gave L.A. values for the successive fractions of L.A. 3.8, 2.9, 3.6, 3.3, and 3.2 respectively (means of five pots). These showed that although there was a slight decline in activity as the volume of leachate increased, the last two fractions were still very active. This

sustained level of activity might have been due to the fact that diffusate concentration is high during the early stages of growth (WIDDOWSON loc. cit.) and that this was the first leaching, so five further pots were leached. These were drawn from a set of older potted plants which had been leached for five weeks up to two days beforehand and had yielded diffusate of approximately L.A. 3.2 each time. The successive mean L.A. values obtained from the successive fractions were L.A. 3.1, 2.9, 2.8, 2.7, 2.8 and 2.5 respectively. The first 40 ml. was thus the most valuable but the test indicated that very large volumes of water were required to leach out all the hatching factor present in the pot. To estimate more precisely the practical limits of diffusate collection, five more pots were selected from a stock of 250 newly planted potatoes and leached as in the last experiment but thrice weekly and for six weeks.

Diffusate was collected in bulk from the 245 pots at 50 ml. per pot and a small sample of this was retained for assay on each occasion for

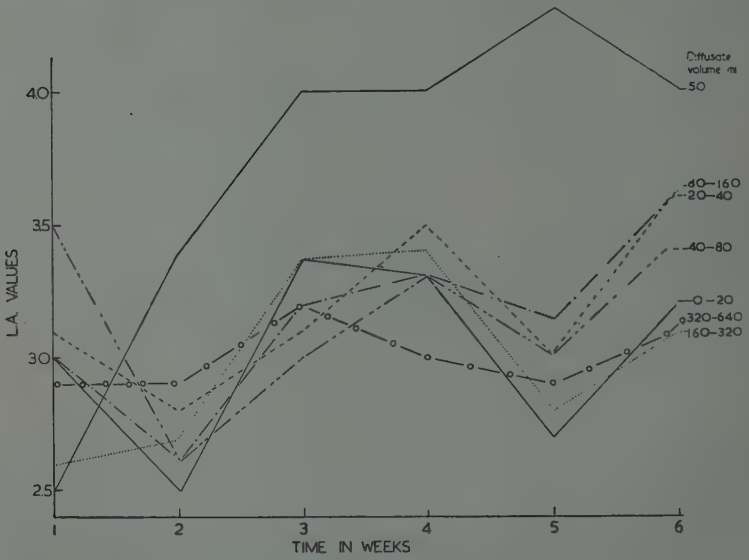

Fig. 1. To show the effect of taking large volumes of diffusate (leachate) from a 6½ inch pot.

comparison with the fractions from the five pots. All the pots were leached eighteen times in all and, after assaying the samples individually, mean L.A. values were computed from each fraction for each week.

These are plotted against time in Figure 1, together with the mean weekly L.A. values of the diffusate collected in bulk. There was little difference between the activity of the successive diffusate fractions, increasing the volume from 20 ml. to 640 ml. being without effect up to six weeks. After the first week higher activities were obtained from the pots leached with 50 ml. of water, the mean L.A. over the whole period being approximately L.A. 4.0 compared with L.A. 3.0 probably because the heavy watering quickly removed the accumulated hatching factor, only that produced between leachings then being available. The approximate mean L.A. values for the limited and the excessive watering were L.A. 4.0 and 3.0 respectively corresponding to a decrease of $^1/_{10}$ in the concentration of hatching factor for the increase of roughly 10 times in volume. This indicates that there is little justification for taking more than 50 ml. of leachate per $6^1/_2$ inch pot since this volume effectively removes a very high proportion of the hatching factor present.

Later, this effect was confirmed when fifty potted potato plants were divided into two sets of twenty five, 50 ml./pot of leachate being taken from one set and 200 ml. per pot from the other, for six weeks. Leaching was started eight days after breakthrough and the L.A. values obtained are plotted against time in Fig. 2.

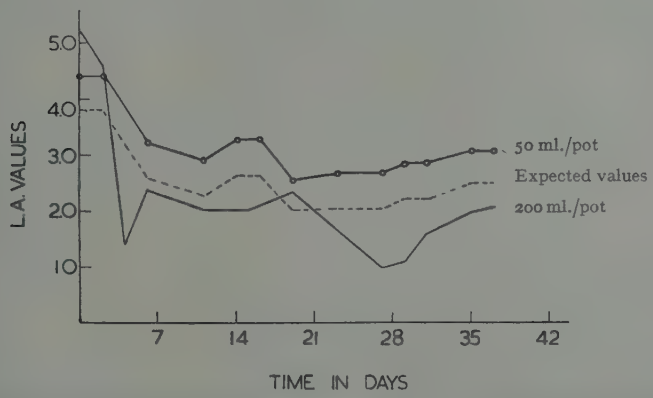

Fig. 2. Comparing the effect of taking 50 ml. and 200 ml. of diffusate (leachate) per pot.

The heavy watering leached out more hatching factor on the first occasion, but later samples were less active than those from the restricted watering and somewhat lower than was to be expected if

the same quantity of diffusate was dissolved in the larger volume of water. The expected L.A. values of the 200 ml. samples calculated on this basis are shown as a broken line on the graph. A comparison of the mean L.A. values over 6 weeks showed that, for the four-fold increase in volume with heavy watering, there was a mean drop of 0.5 L.A. units and approximately $^1/_3$ in the concentration of hatching factor, which confirmed the earlier result.

The suitability of the loam and sand as potting medium was tested against coarse horticultural sand only, some of the plants grown in sand being fed with a nutrient solution and some watered with distilled water only, to obtain a diffusate low in impurities. The nutrient solution contained Calcium nitrate 1.69 g., Sodium dihydrogen phosphate 0.194 g., Potassium sulphate 0.335 g., Magnesium sulphate 0.505 g., Boric acid 0.00545 g., and Manganous sulphate 0.00396 g., per litre. Iron as 0.05 % Ferrous sulphate was sprayed on to the foliage of the plants receiving nutrients, after each leaching. Twenty five plants were grown in each treatment.

Eighteen leachates resulted from this experiment and these were assayed in one series; the mean weekly L.A. values and final root weights (means of five plants) are set out in Table I.

TABLE I

L.A. values of diffusate from potatoes grown in soil and sand

	Soil	Sand		
Time in weeks		With nutrient	No nutrient	Distilled Water
1	4.6	3.4	3.4	2.1
2	3.6	3.3	3.2	2.8
	3.6	3.2	3.2	3.1
4	4.0	1.9	1.9	2.5
5	3.4	2.4	2.5	2,3
6	4.1	2.4	2.6	2,3
Mean L.A.	3.9	2.8	2.8	2.5
Root Weights	30.9	25.2	22.3	19.4

The most vigorous growth and diffusate output was made by the plants growing in soil, which were taller and showed no sign of dying back before six weeks. The addition of 50 ml. of the nutrient solution, to each pot of the second series after each leaching, improved the colour and height of the haulm compared with the other sand series but did not increase the quality of the diffusate. The distilled water series

gave the lowest yields. Soil was considered to be the most satisfactory medium for growing potatoes for diffusate collection in $6\frac{1}{2}$ in. pots in the glasshouse, possibly because of its greater water holding capacity which tended to maintain a more stable temperature.

THE STORAGE OF ROOT DIFFUSATE

The activity of "fresh" diffusate has been found to decline very rapidly during the first two months of storage at 2-3° C. The rate of breakdown then becomes slower and a high proportion of the remaining activity is retained up to approximately two years. A stock of fourteen litres of diffusate collected over a period of four weeks from newly planted potatoes gave an L.A. value of 4.7; it was then divided into seven samples which were stored individually at 3° C. After approximately one month each sample was assayed and gave L.A. values of 3.6, 4.3, 3.1, 4.4, 3.6, 5.1 and 3.7, a greater variability than would be expected. It is therefore advisable to assay individual samples of diffusate as they are collected and later to pool those of suitable activity. The rate of breakdown of single stocks of diffusate collected over a period of about one month and then stored in a carboy at 3° C over two separate years, was estimated by making weekly assays on small samples withdrawn from the carboys. These were tested in a minimum of six dilutions, usually 1/1, 1/4, 1/16, 1/64, 1/256 and 1/1,024 and the mean L.A. values for each month of storage are given in Table II.

TABLE II

The decline in activity of stored potato root diffusate

Time after collection in months	1	2	3	4	5	6	7	8	9	10	11	12
1954	3.8	—	3.0	3.0	2.9	2.9	2.8	3.2	2.5	3.0	3.0	2.3
1955	3.6	—	3.2	3.5	3.7	2.8	3.3	3.4	3.3	3.8	3.5	3.2

DISCUSSION

Measurement of the output of diffusate by potatoes growing in $6\frac{1}{2}$ in. pots containing 3 parts of loam to one part of sand showed that at least 50 ml. of active diffusate can be taken thrice weekly for about six weeks and that there was no increase in the yield of hatching factor if more water was put through the pot. Pots should be saturated and after a short period flooded with 50-100 ml. of water for a yield of diffusate of high activity.

Stronger diffusate was obtained from potatoes growing in soil than in sand, the addition of a standard nutrient solution to plants growing in sand did not increase their diffusate production to the same level. Diffusate production by potatoes grown in sand without nutrients and also in a very fine silica sand was inferior to production in soil. It is recommended that one stock of diffusate adequate for all requirements be collected and stored in bulk each season, because of the variable rate of breakdown of samples stored individually.

ZUSAMMENFASSUNG

Kartoffeln der Sorte Arran Banner, die in 16,5 cm weiten Töpfen wuchsen, wurden Wurzeldiffusate mit unterschiedlichen Wassermengen entzogen, um festzustellen, welche Fraktion des Diffusats die höchste Konzentration des Aktivierungsstoffe enthält. Zwischen den aufeinanderfolgenden Fraktionen wurden bis zu 640 ml nur geringe Abweichungen gefunden. Je 50 ml Wurzeldiffusat je Topf dreimal wöchentlich 6 Wochen lang gesammelt ergaben die günstigste Kombination von Volumen und Konzentration. Kartoffeln, die in einer Mischung von drei Teilen nicht sterilisierten Lehms zu einem Teil Sand wuchsen, brachten eine höhere Ausbeute an Aktivierungsstoff als solche, die in Sand wuchsen und mit einer Standard-Nährstofflösing ernährt wurden.

Diffusate wurden in mehreren kleinen und in wenigen grossen Gefässen bei 3° C aufbewahrt. Einjährige Versuche mit diesen zeigten, dass der Abbau bei einer Aufbewahrung in kleinen Mengen schneller erfolgte.

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Received for publication: November 27, 1957

Nematologica III (1958): 179-183. E. J. Brill, Leiden

CELLULASE AND CHITINASE IN PLANT NEMATODES

BY

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In the course of a survey of the distribution of cellulase and chitinase in soil fauna and flora, some nematodes including pathogenic species were examined, since the presence of these enzymes might throw some light on the mechanism of their initial attack on vascular plants or fungi. Choice of the organisms used was dictated by their availability in sufficient quantity for the preparation of enzyme extracts in convenient bulk and concentration. The material used was analysed for a number of constituents.

MATERIALS AND METHODS

Suspensions of eelworms in Aretan [1]) were supplied by members of the Nematology Department at Rothamsted. On receipt they were washed repeatedly by decantation and a thick suspension was examined microscopically to ensure absence of visible contamination with fungi, debris or dead organisms. After thorough washing, the suspensions were centrifuged, giving a thick paste of organisms (dry matter 9-13 %). The suspension was transferred with a spatula, a little at a time, to the ground surface of a B24 stopper. The stopper fitted into a standard socket that had been sealed with a flat floor 5 mm below the lower margin of the ground surface. The paste of organisms was ground between the surfaces of the stopper and socket, drops of water being added when necessary to wash the ground material into the base of the grinder. Slow careful pressure and twisting was used to avoid as far as possible any rise in temperature during grinding. After the paste had all passed between the surfaces, the thick slurry in the bottom of the grinder was washed into a tube and centrifuged. After decantation, the deposit was ground a second time. Two passages ensured that no intact eelworms could be seen in the suspension. Extracts were preserved, when necessary, with the addition of a few drops of toluene at 5° C.

1) Bayer Products: a 3 % solution of an organic mercurial compound.

Methods used for the detection of chitinase activity were those described earlier (TRACEY, 1955a). Cellulase activity was detected by following the production of reducing sugar at pH5 from a suspension of finely divided cellulose precipitated by dilution of a cellulose dispersal in syrupy phosphoric acid. Methods for the detection and determination of chitin (TRACEY, 1955b) and for the determination of total carbohydrate and nitrogen (HOLDEN, PIRIE & TRACEY, 1950) have also been published elsewhere. Reducing sugar was determined by NELSON's (1944) colorimetric modification of the SOMOGYI method.

RESULTS

Analytical results for a sample of *Ditylenchus dipsaci* (Kühn) Filipjev will be given as typical of a number of samples of this organism and of *D. destructor* Thorne and *D. myceliophagus* Goodey. One gram of dry matter represents 3-4 $\times$ 10^7 eelworms. Of the total dry matter, 40 % was water soluble, 33 % chloroform soluble and 27 % insoluble in water or chloroform. The high proportion of lipid material, reflected in the figures for the chloroform soluble fraction, was also evident from the formation of the coherent fatty material at the surface when the extracts were centrifuged; 68 % of this material was soluble in chloroform and it contained 85 % of the total chloroform soluble material. The dry matter of intact worms contained 6.4-6.6 % N (about 40 % 'crude protein') of which half was water soluble. Of the water soluble nitrogen, 40 % was not precipitated by 5 % trichloracetic acid. Of the total dry matter 10 % appeared to be carbohydrate as judged by the orcin method, 85 % of it appearing in the water soluble fraction and accounting for a fifth of the dry weight of this fraction. Half the total water soluble carbohydrate was accounted for as reducing sugar calculated as glucose. Chitin was absent from *Ditylenchus dipsaci* and from larvae of *Heterodera rostochiensis* Wollenweber or, if present, accounted for less than 0.4 % of their total dry matter.

H. rostochiensis egg cases and cysts were also examined for chitin. Preparations containing opened cysts, eggs and larvae (CEL) and eggs and larvae only (EL) were dried and analysed after hydrolysis in 6N HCl. Portions of the preparations were also treated with sodium chlorite solution and then with chitinase (TRACEY, 1955b). The EL preparations consistently had a higher chitin content by both methods and it is probable therefore that the cysts contained no chitin, or negligible amounts and that, since adult worms and larvae contained

very little or no chitin, the chitin found may be attributed to the egg cases alone. The direct chemical method of hydrolysis using unpurified material may lead to spurious positives owing to the presence of materials other than chitin that yield glucosamine on hydrolysis. The finding, therefore, that chitin was present by the enzymic assay after treatment with sodium chlorite is the more reliable (TRACEY, 1957). The chitin digested by chitinase accounted for 27 % of the total calculated after acid hydrolysis in each preparation, indicating a single source of chitin and giving added support to the conclusion that chitin is present solely in the egg cases of *H. rostochiensis* and not in the larvae or cysts.

Extracts of *Turbatrix aceti* (Müller) subsp. *aceti* Peters, *Ditylenchus dipsaci* (a race from oats), *D. destructor* (from potatoes) and *D. myceliophagus* (from mushrooms) were examined for the presence of cellulase and chitinase. The extracts were normally prepared from about a gram of packed organisms (about 100 mg dry matter containing 3-4 $\times$ 10^6 organisms) and diluted to a final volume of 5 ml. Neither cellulase nor chitinase activity was detected in extracts of *Turbatrix aceti*. Both enzymes were present in extracts of all three species of *Ditylenchus*. Cellulase and chitinase could not be detected in the water in which heavy suspensions of the eelworms had been kept overnight at 5° C.

Chitinase

The extracts with chitinase activity degraded chitin to acetylglucosamine at a constant rate for periods of up to 12 days when incubated at 37° in the presence of finely divided *Sepia* chitin. Assays of activity were carried out using 1 ml extract (from 15-25 mg dry weight of eelworms), 1 ml chitin suspension (10 mg) and 2 ml pH 5, 0.2 M acetate buffer in a total volume of 5 ml. A few drops of toluene were added to discourage bacterial growth. *D. destructor* and *D. myceliophagus* had similar activities (2.3 mg (*destructor*) and 2.9 mg (*myceliophagus*) chitin degraded to acetylglucosamine/day/g dry matter of eelworms) and the activity of extracts of *D. dipsaci* were greater by a factor of 1.5. Dialysis of the extracts led to extensive precipitation of material and loss of enzyme activity. In each extract, the loss was 80 % and the ratio of activity between the two species remained the same. The activity of dialysed *D. destructor* extract was increased by a factor of 2.75 by added protein and of dialysed *D. dipsaci* extract by a factor of 2 (TRACEY, 1955a). The pH optimum of the *D. dipsaci*

enzyme was in the region of 5.0, rather less degradation of chitin being found at pHs 4.5 and 5.5 than at that pH. At pH 7 activity was reduced to half that at pH 5.

Cellulase

The rate of cellulose degradation to reducing sugar by the extracts fell of rapidly with time of incubation and this, together with high blanks, rendered quantitative comparisons between extracts very difficult. It was observed that the pH optimum of the cellulase of *D. dipsaci* was in the region of 5.0 and the enzyme showed a similar behaviour under changed pH conditions to that of the chitinase. Protein activation of cellulase in dialysed *D. dipsaci* extracts was by a factor of 1.3-1.4. A direct comparison of cellulase activity in dialysed extracts of *D. dipsaci* and *D. myceliophagus* gave values for cellulose degraded in one day per g eelworm dry matter of 12.7 mg and 2.7 mg respectively.

Polygalacturonase

Evidence suggesting that a slow degradation of pectic acid to galacturonic acid occured during incubation with dialysed *D. dipsaci* was got on one occasion.

DISCUSSION

The presence of 33 % of the dry matter as chloroform soluble material and less than 2 % as insoluble polysaccharide indicated the reliance of these organisms on fat as an energy store. The apparent absence of chitin from the adult and larval forms and its presence in the egg cases of *H. rostochiensis* is in agreement with the results of Chitwood (1938) and Rudall (1955) using other nematodes.

The difficulties inherent in the demonstration of the production of a hydrolytic enzyme by a small invertebrate with a gut are many (Tracey, 1958) but the observations described here appear unequivocal. *Turbatrix aceti* is a bacterial feeder but proved to contain detectable amounts of neither enzyme. The three species of *Ditylenchus* used have gullets too small to admit bacteria and have guts free from visible micro-organisms. The enzymes whose presence were demonstrated cannot have been produced by other organisms contaminating the preparations for these were free from gross contaminants visible microscopically and the washings prior to grinding contained no trace of cellulase or chitinase. There seems no doubt therefore that the three species of *Ditylenchus* produce both these enzymes. Although a more active chitinase preparation was secured from *D. myceliophagus* derived

from mushrooms than that *D. destructor* from potatoes, the difference was not enough to be diagnostic. A direct comparison of a *D. dipsaci* extract with an extract prepared by mincing *Helix aspersa*, O. F. Müller showed that the eelworm extracts had 20-40 % of the snail chitinase activity on a total initial dry matter basis.

The cellulase and chitinase of these species of nematode presumably assist in the penetration of the cell walls of vascular plants and those of the fungi. Whether the possession of chitinase by *D. dipsaci* is an indication that it may, under some circumstances, prey on soil fungi or whether it represents an ancestral ability no longer used cannot be decided at present.

I should like to express my thanks to D. W. Fenwick, J. B. Goodey and B. G. Peters of the Nematology Department, Rothamsted, for the provision of the material used in this work. Very brief mention of some of the findings reported here have appeared earlier (Goodey, 1952, Tracey, 1952).

ZUSAMMENFASSUNG

Ditylenchus dipsaci aus Hafer, *D. destructor* aus Kartoffeln und *D. myceliophagus* aus Pilzbeeten zeigten Chitinase- und Cellulase Aktivität. Das pH-Optimum für diese Enzyme liegt in der Nähe von pH 5. Beide Enzyme werden durch zugesetzte Eiweissstoffe aktiviert. Es hatte den Anschein, als ob *D. myceliophagus* eine aktivere Chitinase bildet als *D. destructor*, doch war der Unterschied als ein Unterscheidungsmerkmal nicht ausreichend. In Extrakten von *Turbatrix aceti* wurde weder Chitinase noch Cellulase gefunden. Die Eischalen von *Heterodera rostochiensis* enthielten Chitin, aber nicht die Larven und die Zysten.

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Received for publication: December 19, 1957

Nematologica III (1958): 184-196. E. J. Brill, Leiden

STUDIES ON THE BIOLOGY OF *RADOPHOLUS SIMILIS* (COBB, 1893) THORNE, 1949

Part II. MORPHOLOGICAL VARIATION WITHIN AND BETWEEN PROGENIES OF SINGLE FEMALES

BY

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When *R. similis* specimens are recovered by incubating infected roots according to the method described by YOUNG (1954) and the nematodes are studied under the microscope, a pronounced morphological variation between individuals is often observed. To test the possibility of mixture of species the morphological variations within the progeny of single females were studied. If this latter variation proved to be small and the progenies of different, females could be arranged in groups morphologically different from each other, the probability of a mixture of taxa would become apparent. If, however, the variability within the progeny of a single female was considerable, the great variation between individuals of a naturally occurring population would not be a basis to suspect a mixture of taxa, since it is assumed that the variation between individuals of a naturally occurring population is as great or greater than between individuals produced by a single female.

The progenies of eighteen females were studied. Three progenies resulted from *R. similis* females obtained from an infested citrus grove, which had been used to inoculate sour orange seedlings. Eight progenies resulted from *R. similis* females collected from an infested citrus grove and used to inoculate corn, *Zea mays* L. var. Bantam, and seven progenies were the offspring of seven female burrowing nematodes which were recovered from infected roots of banana, *Musa nana* Lour., and then propagated on corn.

In the smaller progenies all specimens were measured and studied, while twenty females and twenty males were selected at random from the more populous ones.

The nematodes to be measured were stored at 5° C over night. This

put them in a straight, quiescent condition. Measurements were made in 2 % aqueous formalin to the nearest whole micron using an eyepiece micrometer scale. Measurements were recorded as means, ranges, standard deviations from the mean and standard error expressed as percentage of the mean and included: total length, diameter, spear length, distance of the excretory pore from the anterior end, length of tail, anal body diameter, the ratios *a*, *b*, *c* [1]), distance of the vulva from the anterior end as a percentage of the total length in females and length of spicules and gubernaculum in males. In addition to the statistical information, results are reported of studies of the morphological variation within single female progenies.

PROGENY STUDIES OF R. SIMILIS FEMALES COLLECTED FROM CITRUS AND USED TO INOCULATE SOUR ORANGE SEEDLINGS

Fresh sour orange seeds were submerged in water for 30 minutes, soaked in dilute NaOH for 10-15 minutes and thoroughly rinsed in water. The seeds were then treated with 1 % 8-hydroxy-quinoline-sulfate for 1 minute, after which the outer seed coat was removed and the seeds rinsed in dilute sodium hypochlorite ("Chlorox" diluted tenfold with water). Subsequently the seeds were planted on sterile nutrient agar plates containing KH_2PO_4 2.45 g, $Ca(NO_3)_2$ 0.85 g, $MgSO_4$ 3.69 g, Fe citrate trace, and agar 20 g per 500 ml of water. After germination each of forty seedlings was inoculated with one *R. similis* female obtained from an infested citrus grove. Ten days later each seedling was transferred to a 4-inch pot containing a steam-sterilized mixture of soil, peat and fertilizer. An additional forty seeds were treated similarly but not inoculated and served as controls. After three months the results were examined. Each day, over a period of four weeks, the root systems of the inoculated plants and of the controls were incubated for six days. All controls were found to be free of nematodes, and among the inoculated plants only three yielded burrowing nematodes. The latter root systems were then incubated for periods up to twenty-one days. The specimens were collected by shaking the roots in an incubation vial with water and then pouring the washings into a Syracuse dish. The nematodes were mounted in 2 %

1) *a* = length divided by greatest width.
b = length divided by the distance of the base of the median bulb from the anterior end.
c = length divided by length of tail.

formalin and the adults measured. No males were recovered and the number of females was six, six, and five respectively. The results of the measurements are recorded in Table I. All measurements given are in microns unless a ratio is expressed.

TABLE I

Measurements of female R. similis specimens recovered from sour orange inoculated with one female R. similis obtained from an infested citrus grove

	Progeny Number	Length	Diameter	Stylet Length	Position of Excretory Pore from Anterior End	Tail Length
M. [1])	1	690.3	24.2	18.5	94.6	62
R.	1	580-785	22-26	18-19	62-104	49-72
S.D.	1	± 80.77	± 1.79	± 0.77	±18.86	± 7.92
S.E.M.	1	4.8	3.0	1.7	8.1	5.2
M.	2	563	23.5	18	74.6	63
R.	2	511-615	23-24	18	60-87	61-66
S.D.	2	± 40.70	± 0.55	—	± 10.78	± 1.79
S.E.M.	2	2.9	0.9	—	5.9	1.1
M.	3	606	23.4	17.4	80.4	60.2
R.	3	540-697	21-28	17-18	55-99	55-65
S.D.	3	± 58.69	± 2.70	± 0.55	±16.58	± 3.56
S.E.M.	3	4.4	5.1	1.4	9.2	2.6

	Anal Body Diameter	*a*	*c*	Position of Vulva from Anterior End as a Percentage of Total Length	Number of Individuals
M.	18	27.9	11.2	58.8	6
R.	15-21	25.6-30.2	8.5-12.8	56.0-62.5	6
S.D.	± 2.76	± 2.00	± 1.47	± 2.17	6
S.E.M.	6.2	2.9	5.4	1.5	6
M.	16.3	23.9	8.9	55.9	6
R.	16-17	21.3-25.6	8.0- 9.9	52 -58.4	6
S.D.	± 0.52	± 1.59	± 0.49	± 2.39	6
S.E.M.	1.3	2.7	2.2	1.8	6
M.	15.6	26.1	10.1	57.1	5
R.	11-19	23.5-29	9 -11.4	52.5-59.0	5
S.D.	± 3.43	± 2.08	± 1.13	± 2.88	5
S.E.M	9.8	3.6	5.0	2.2	5

1) M. = Mean.
R. = Range.
S.D. = Standard deviation from the mean.
S.E.M. = Standard error expressed as a percentage of the mean.

PROGENY STUDIES OF R. SIMILIS FEMALES COLLECTED FROM CITRUS AND USED TO INOCULATE CORN

Corn seeds were germinated under sterile conditions in petri dishes containing moist sand. After the roots were about one inch long a series of individual plants was inoculated by placing a single female *R. similis,* obtained by incubating infected citrus roots (*Citrus sinensis* Osbeck, sweet orange on *Citrus limon* Burm., rough lemon rootstock), on the root close to the tip. Two days later twenty-two inoculated corn seedlings and twenty-two non-inoculated controls were planted in steam sterilized soil and maintained in the greenhouse for two months. The soil temperatures around the corn roots during this time ranged from 74-85° F mostly 78° F. At the end of the two months' period the plants and surrounding soil were examined for burrowing nematodes by employing the modified Baermann funnel technique as described by Christie & Perry (1951), and by incubating the roots. Eight out of the twenty two inoculated corn plants yielded burrowing nematodes both from the soil and from the roots, two yielded one single *R. similis* from the soil and in the remaining twelve no burrowing nematodes were found. The controls were all free of nematodes. Counts of recovered female, male and larval *R. similis* specimens as well as the total number from each corn plant are listed in Table II.

Table II

Number of R. similis specimens recovered after two months from corn plants inoculated with one female burrowing nematode obtained from an infested citrus grove

Inoculation Number	Number of recovered *R. similis*			Total Number
	Female	Male	Larvae	
C1	12	2	21	35
C2	1	—	—	1
C3	9	5	32	46
C4	113	32	332	477
C5	—	1	—	1
C6	81	16	201	298
C7	96	25	238	357
C8	84	36	332	452
C9	21	11	47	79
C10	73	19	234	326
C11-22	0	0	0	0
Controls:				
C23-44	0	0	0	0
Not inoculated				

Whenever possible twenty male and twenty female specimens were measured from each of the progenies listed in Table II. Table III

TABLE III

Measurements of female R. similis specimens recovered from corn roots inoculated with one female R. similis obtained from an infested citrus grove

	Progeny Number	Length	Diameter	Stylet Length	Position of Excretory Pore from Anterior End	Tail Length
M.	C7	596.0	19.9	17.9	89.4	62.8
R.	C7	458-720	17-27	16-20	67-107	49-75
S.D.	C7	± 73.88	± 2.7	± 1.08	± 10.05	± 6.85
S.E.M.	C7	2.8	3.0	1.3	2.5	2.4

	Anal Body Diameter	*a*	*b*	*c*	Position of Vulva from Anterior End as a Percentage of Total Length	Number of Individuals
M.	15.0	30.0	9.3	9.3	56.5	20
R.	12-18	26.0-34.3	8.2-10.4	8.5-10.5	53.9-58.7	20
S.D.	± 1.73	± 2.67	± 1.83	±0.62	± 1.58	20
S.E.M.	2.6	2.0	4.4	1.5	0.6	20

TABLE IV

Measurements of male R. similis specimens recovered from corn roots inoculated with one female R. similis obtained from an infested citrus grove

	Progeny Number	Length	Diameter	Stylet Length	Position of Excretory Pore from Anterior End	Tail Length	Anal Body Diameter
M.	C7	563.1	18.3	13.1	89.9	63.5	12.2
R.	C7	447-653	14-21	12-14	76-104	51-71	11-14
S.D.	C7	± 53.6	± 1.84	± 0.72	± 7.18	± 5.8	± 2.95
S.E.M.	C7	2.1	2.2	1.2	1.7	2.1	5.3

	Spicules	Guberna-culum	*a*	*b*	*c*	Number of Individuals
M.	17.3	8.5	33.3	9.2	7.9	21
R.	16-19	7-10	29.9-40.4	8.2-10.0	7.7-11.4	21
S.D.	± 1.06	±0.79	± 3.27	±0.6	±1.29	21
S.E.M.	1.3	2.0	2.1	1.4	3.6	21

shows measurements of twenty female specimens from one such progeny, while Tabel IV contains the measurements of twenty-one male specimens from the same progeny. The data for all the other progenies, not published here to save space, are available on request.

PROGENY STUDIES OF R. SIMILIS FEMALES COLLECTED FROM BANANA AND USED TO INOCULATE CORN

Burrowing nematodes obtained by incubating infected banana roots, *Musa nana*, were used to inoculate corn as described in the previous experiment. Each of ten corn plants were inoculated with one female *R. similis*. After two months, six out of the ten corn plants supported a good population of burrowing nematodes. Only one male and one female were recovered from an additional corn plant. The remaining three plants were free of nematodes. Ten non-inoculated controls were also free of nematodes. The recovered populations were rather small, varying from two to 187 specimens per plant, mostly females and larvae. Tables V and VI respectively show the various measurements of twenty female and fourteen male burrowing nematodes from one progeny recovered by incubating the corn roots and examining the surrounding soil using the Baermann funnel technique. Here again the data for the other progenies are available on request.

Table V

Measurements of female R. similis specimens recovered from corn roots inoculated with one female R. similis obtained from infected banana roots

	Progeny Number	Length	Diameter	Stylet length	Position of excretory pore from anterior end	Tail length
M.	B2	613.8	24.6	18.3	97.1	63.2
R.	B2	490-688	16-33	18-19	87-105	54-70
S.D.	B2	± 60.33	± 5.22	± 0.48	± 6.43	± 5.49
S.E.M.	B2	2.2	4.7	0.6	1.5	1.9

	Anal body diameter	*a*	*b*	*c*	Position of vulva from anterior end as percentage of total length	Number of individuals
M.	16.8	25.7	9.4	9.0	56.5	20
R.	13-21	20.0-30.6	8.2-10.5	8.7-10.4	53.6-59.2	20
S.D.	± 2.63	± 3.24	±2.51	±2.01	± 1.97	20
S.E.M.	3.5	2.8	6.0	4.7	0.8	20

Table VI

Measurements of male R. similis specimens recovered from corn roots inoculated with one female R. similis obtained from infected banana roots

	Progeny number	Length	Diameter	Stylet length	Position of excretory pore from anterior end	Tail length
M.	B2	552	15.3	12	91	64.3
R.	B2	510-580	15-16	11-13	89-92	51-72
S.D.	B2	± 36.87	± 0.58	± 1.0	± 1.73	±11.56
S.E.M.	B2	1.8	1.0	2.2	0.5	4.8

	Anal body diameter	Spicules	Gubernaculum	*a*	*b*	*c*	Number of individuals
M.	13.7	15	9	36.1	10.1	8.7	14
R.	13-15	14-17	8-10	31.9-38.7	9.4-10.5	7.8-10.0	14
S.D.	± 1.15	±1.3	±0.6	± 1.15	± 0.87	±1.15	14
S.E.M.	2.2	2.3	1.8	0.8	2.3	3.5	14

DISCUSSION

Morphological variation in general and between progenies of single females. A detailed description of *Tylenchus similis* based on specimens from diseased banana tissues (*Musa sapientum*) was published by Cobb in 1915. Thorne (1949) in studying specimens from sugarcane (*Saccharum officinarum*) and pepper (*Piper nigrum*) pointed out differences from Cobb's description, namely, the number of head annules being three in his specimens instead of eight to ten as stated by Cobb and the three oesophageal nuclei being in one elongated lobe rather than in three separate lobes.

In the Florida specimens we studied the oesophageal glands were in three separate lobes. The number of head annules was very hard to determine. Mostly six or seven annules could be distinguished, but not infrequently only three or four were seen with certainty. In this latter case, however, the head annules were rather wide and each one might well represent two or more of the smaller ones.

No significant morphological differences were detected between the progenies of female burrowing nematodes collected from banana and citrus. The gross appearance of a *R. similis* population on banana sometimes gave a different overall impression than the one on citrus; however, this "difference" disappeared after culturing nematodes from both origins on corn.

Although some progenies consisted of large animals, others of smaller ones, overlapping of sizes was always considerable and no significant differences were present between any two progenies.

Morphological variation within the progenies of single females. As is shown in Tables I, III, IV, V, and VI the differences in size, etc., of individuals within the progeny of a single *R. similis* female were very great. To be able to compare the values of the different measurements the standard error was calculated for every series of measurements and expressed as percentage of the mean (S.E.M.). These S.E.M. values show that the position of the vulva from the anterior end, when expressed in percentage of the total length, is a good diagnostic character since these values rarely exceeded 1.0. This is also true for the female stylet length. The S.E.M. values for the diameter and the anal body diameter were very great, mostly around 4.0, while the values for total length and the ratios *a*, *b*, and *c* ranged primarily between 2.0 and 3.0. Not all morphological variability is readily expressed in figures and tables, however. Additional observations are therefore summarized below.

Females.—The head measured mostly 9 μ wide by 4 μ long, but varied from 7.5 μ wide by 3.2 μ long to 10 μ wide by 5 μ long (Fig. 1D and 1E). The number of head annules was very hard to determine, but varied from six or seven annules to only three or four. Where the head annules were few they were wider and each annule might represent two or more of the smaller ones. In some progenies the outer margins of the cephalic framework of most individuals were prominent and extended somewhat posteriorly, but in other progenies there were always a few specimens also showing this character.

As is stated above, the female stylet length is one of the most reliable characters; however, it varied from 16 μ to 20 μ within a given progeny (Fig. 1D and 1E). The stylet knobs varied somewhat in appearance, mainly due to different views of observation (Fig. 1C, 1D, and 1E). Some progenies had more rounded knobs than others. The knobs ranged in size from 2 μ by 3 μ to 3 μ by 4 μ (Fig. 1D and 1E). In some progenies a small spherical object was observed about 1 μ in diameter, situated on or very close to the stylet, 1-2 μ in front of the stylet knobs (Fig. 1C). When present in a progeny 5-10 % of the individuals showed this character and its absence in the other specimens could not be explained as due to a certain observational view. In rare instances the stylet tip was abnormally enlarged, all of it refractive (Fig. 2A). One progeny had seven specimens showing this character.

The excretory duct opened ventrally 1-2 μ behind the hemizonid. The posterior part of the excretory duct was in most individuals of a progeny situated on the left side of the body, but all of seven progenies examined for this purpose showed 5-15 % of the specimens with the posterior part of the excretory duct on the right side of the body.

The vulval lips were usually not or very slightly protruded. In some cases, one a young female still within its final molt, a pronounced protrusion of the vulval

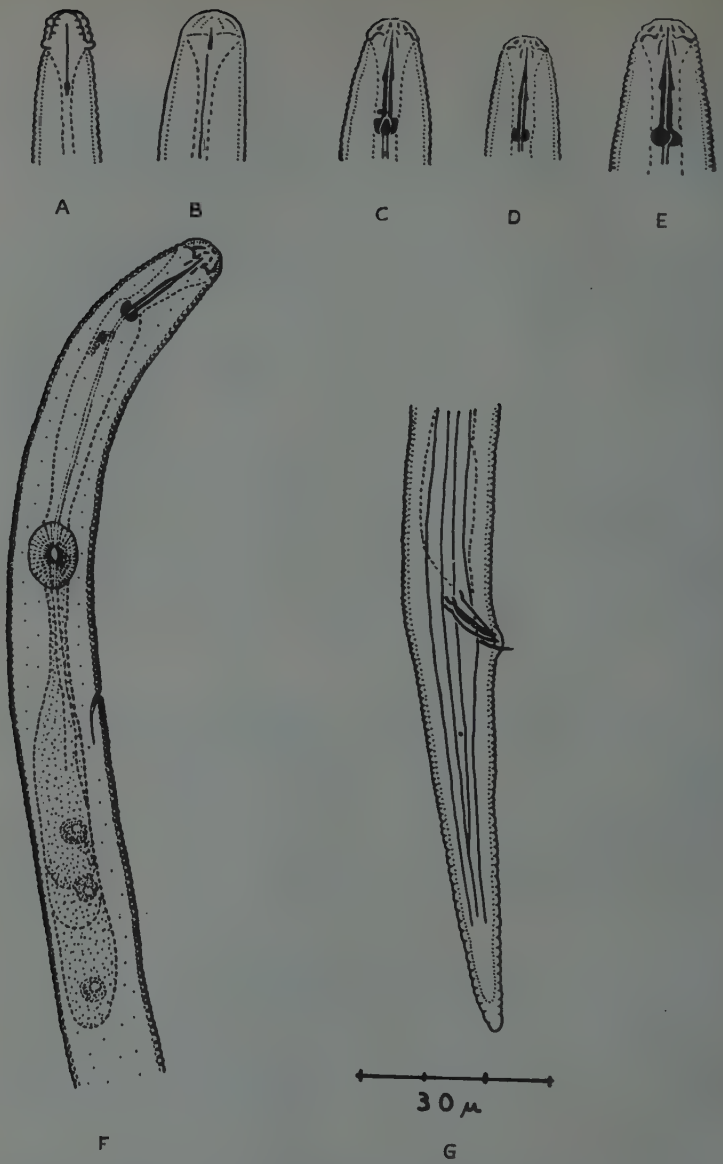
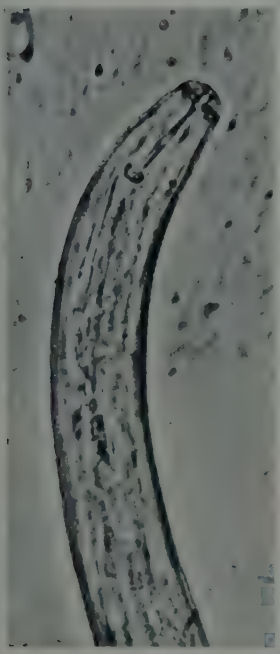


Fig. 1.—Variation within the progeny of a single female *R. similis*. Camera lucida drawings. A: commonly occurring male head, 8 μ wide by 5 μ long. B: rarely occurring male head, 11 μ wide by 4 μ long. C: female head 9 μ wide by 4 μ long with an additional spherical object on stylet. Stylet 18 μ long, stylet knobs 2.5 μ by 3.5 μ. D: small female head 7.5 μ wide by 3.2 μ long. Stylet 16 μ long, stylet knobs 2 μ by 3 μ. E: large female head 10 μ wide by 5 μ long. Stylet 20 μ long, stylet knobs 3 μ by 4 μ. F: anterior end of abnormal male G: posterior end of abnormal male. A-E all from one progeny. F and G from one other progeny.

A



B

C

Fig. 2—Variation within the progeny of a single female *R. similis*. Photomicrographs. A: female with abnormally enlarged, refractive stylet tip (magnification 1164 ×). B: anterior end of abnormal male (magnification 776 ×). C: posterior end of abnormal male (magnification 776 ×). A from one progeny. B and C from one other progeny.

lips was observed. In some progenies protrusion of the vulval lips was far more common than in others. The gonads were usually outstretched and varied considerably in length, with the larger animals not necessarily having the larger gonads. They ranged from 110 μ to 226 μ for the anterior gonads and from 105 μ to 270 μ for the posterior ones. Generally the anterior gonads were longer than the posterior ones. In case the gonads were very long for a particular female they would be bent or more rarely, reflexed.

Variation in shape and size of the tails is shown in Fig. 3A to 3E. The tail shown in Fig. 3B was interesting in that the lateral ridges went around the tip causing a dorso-ventral division of the tail. Several progenies had one or two specimens showing these split tails, and they occurred in both males and females. The split tails were much shorter than the average tail length.

Aereolation of parts of the lateral fields was observed within some progenies. In one progeny it was observed very plainly. A co-worker was called over to verify the fact. However, by the time a second co-worker took his place at the microscope the phenomenon had disappeared. Since then temporary aereolation of the lateral fields has been observed several times, but occasionally the aereolation did not disappear, at least not readily, and seemed to be of a permanent nature. An artifact as a result of the fixation in formalin seems to be the most logical explanation of these aereolations.

Males.—The size of the head varied from 4 μ long by 6 μ wide to 5.5 μ long by 8 μ wide in some progenies, from 5 μ long by 7 μ wide to 6 μ long by 8.5 μ wide in others. Its shape varied considerably from rarely almost spherical to box shaped, always wider than long. Usually the head was wider at the first head annule. Anterior to this first annule the head was of uniform, but smaller diameter, or tapered very slightly (Fig. 1A). The head was always set off by a constriction except in one case, where the head was continuous with the body, rounded off and with very little if any striation (Fig. 1B).

The stylets varied from almost non-existing to rather well developed. As in the females most male specimens had the posterior part of the excretory duct on the left side of the body, but about 10 % of a progeny had it on the right side.

Male gonads were outstretched, very rarely reflexed, ranging from 168 μ to 223 μ in length.

The size of the spicules ranged from 14 μ to 18 μ in one progeny, from 17 μ to 20 μ in another, but was fairly constant for most individuals within a progeny. This is also true for the gubernaculum, although a range from 8-11 μ was observed (Fig. 3F to 3I).

The shape of the tails within a progeny varied greatly from narrow, pointed tail tips (Fig. 3G) through a more usual, wider tip (Fig. 3F) to a rarely occurring, abnormally wide tip (Fig. 3I). Very few males had much shortened tails with a dorso-ventral split (Fig. 3H).

OCCURRENCE OF AN ABNORMAL MALE

Among the progeny of one female an abnormal individual was encountered, photomicrographs of which are shown in Fig. 2B and 2C, and camera lucida drawings in Fig. 1F and 1G. Its length was 497μ, diameter 15 μ. The head was internally sclerotized like a fourth stage larva and measured 6.5 μ wide by 3.7 μ long. The number of annules on the head could not be determined with certainty. The stylet was

strong, 16 μ long, with strong knobs measuring 2.7 by 3 μ, projecting somewhat anteriad. The dorsal gland orifice was 3.2 μ posterior to the stylet knobs. The median bulb measured 12 μ long by 9 μ wide and its base was 63 μ from the anterior end. The excretory pore was situated 84 μ from the anterior end. Three distinct oesophageal glands with three large nuclei were present and measured 71 μ to the base of the median bulb. The testis was well developed, 144 μ long. Spicules and gubernaculum were rather small but well developed and measured 13 μ and 7 μ, respectively. No caudal alae were present but the lateral fields widened somewhat at the position of the spicules (Fig. 1G). The tail measured 64 μ, the anal body diameter 14 μ. The phasmids were small

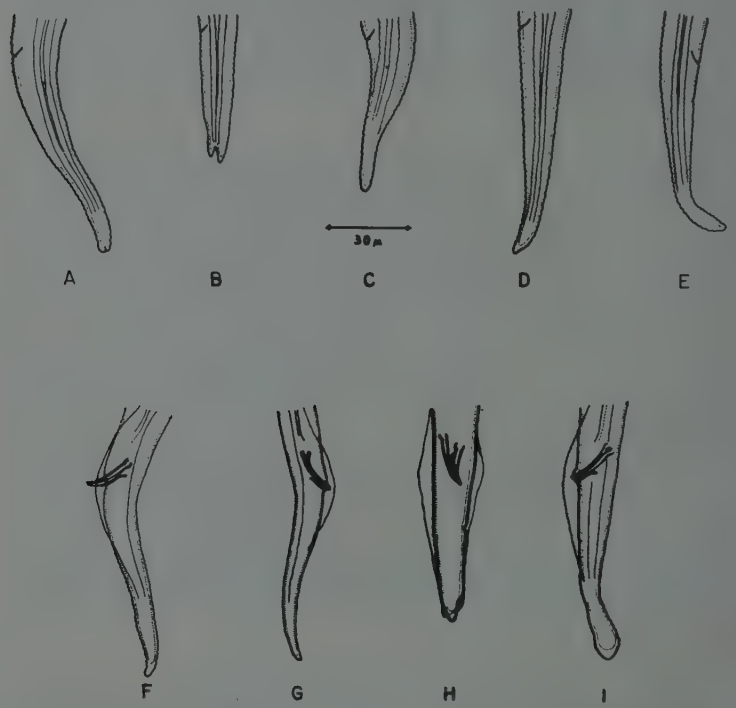


Fig. 3.—Variation of female (A-E) and male (F-I) tail tips within the progeny of one female *R. similis*. A: tail 79 μ long, A. B. D. (anal body diameter) 17 μ. B: tail 42 μ long, A. B. D. 15 μ. C: tail 55 μ long, A. B. D. 17 μ. D: tail 75 μ long, A. B. D. 19 μ. E: tail 63 μ long, A. B. D. 15 μ. F: tail 70 μ long, A. B. D. 13 μ, spicules 19 μ, gubernaculum 11 μ. G: tail 64 μ long, A. B. D. 12 μ, spicules 17 μ, gubernaculum 8 μ. H: tail 51 μ long, A. B. D. 13 μ, spicules 18 μ, gubernaculum 9 μ. I: tail 68 μ long, A. B. D. 15 μ, spicules 19 μ, gubernaculum 10 μ.

and dot like and were situated 41 μ from the tail tip. The lateral fields were 5 μ wide, the two outer ones being slightly wider than the middle one. Striae were less than 1 μ wide. $a = 33.1$; $b = 7.9$; $c = 7.8$.

Noticing the striking similarity of this abnormal specimen to a fourth stage larva it is believed that we here dealt with a case of neoteny.

ACKNOWLEDGMENTS

The author is indebted to Dr B. G. Chitwood, Chief Nematologist, State Plant Board of Florida, and to Dr. G. F. Weber and Mr W. H. Thames, Department of Plant Pathology and Department of Soils, University of Florida, respectively, for helpful advice and criticism of the manuscript. Mrs Jean Smith, State Plant Board of Florida, took the photographs.

ZUSAMMENFASSUNG

Versuche Setzlingen von sauren Apfelsinen (*Citrus aurantium* L.) mit je einem einzelnen weiblichen *Radopholus similis* zu infizieren, das aus einem parasitierten Citrus-Garten gesammelt worden war, verliefen in 3 von 40 Fällen erfolgreich. Nach 3 Monaten betrug die Zahl der wiedergefundenen Älchen 6, 6, und 5; alle waren weiblich. Infektionsversuche von Maispflanzen (*Zea mays* L. var. bantam) mit je einem einzelnen weiblichen *R. similis*, gesammelt aus demselben parasitierten Citrus-Garten waren in 10 von 22 Versuchen erfolgreich. Nach 2 Monaten wurden bis zu 477 *R. similis* Individuen an einer Maispflanze wiedergefunden. Infektionsversuche von Maispflanzen mit je einem einzelnen weiblichen *R. similis*, gesammelt von parasitierten Bananenwurzeln (*Musa nana* Lour.) waren in 7 von 10 Fällen erfolgreich. Nach 2 Monaten hatten die Maispflanzen 2 bis 187 Älchen aufzuweisen. Keine bedeutenden Unterschiede wurden zwischen der Älchennachkommenschaften von Banane und Citrus gefunden. Die Variationen innerhalb der Nachkommenschaft der einzelnen weiblichen *R. similis* waren sehr gross. Stachellänge der Weibchen und Lage der Vulva, ausgedrückt in Prozenten zur Gesamtkörperlänge, sind wertvolle Merkmale. Gesamtkörperlänge, Körperbreite, Analbreite und in geringerem Masse auch *a*, *b*, und *c* sind sehr veränderlich. Die Weibchen einer Nachkommenschaft variierten in der Grösse der Köpfe, der Zahl der Kopfringe, der Lage des Exkretionsporus, des Vorstosses der vulvalen Lippen sowie in Form und Grösse der Gonaden, und des Schwanzes. Ein kleines kugeliges Gebilde war bisweilen bei oder sehr nahe an der Basis des Stachels vorhanden. Die Stachelspitze war mitunter unnatürlich vergrössert. Der Exkretionsporus liegt meistens an der linken Seite des Körpers, aber in 5-15 % der Individuen an der rechten Seite. Eine Spaltung des Schwanzes kommt bisweilen vor. Querringelung der Seitenfelder war vorübergehend sichtbar. Männchen zeigten eine grosse Verschiedenheit in Form und Grösse der Köpfe, Zahl der Kopfringe, Stachelentwicklung, Lage des Exkretionsporus und Form des Schwanzes. Spicula und Gubernakulum waren in Form und Grösse ziemlich konstant. In der Nachkommenschaft eines Weibchens trat ein Männchen auf, das einer Larve vierten Stadiums mit einem sklerotisierten, gerundeten, nicht abgesetzten Kopf und einem gut entwickelten Stachel mit Stachelknöpfen sehr ähnlich war, aber einen gut entwickelten Hoden, Spicula und Gubernakulum, jedoch keine kaudale Alae aufzuweisen hatte.

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Received for publication: January 11, 1958

Nematologica III (1958): 197-204. E. J. Brill, Leiden

PURIFICATION AND PROPERTIES OF EELWORM HATCHING FACTORS

Hatching Factors for the Cabbage, Hop and Beet Root Eelworms (*Heterodera cruciferae, H. humuli* and *H. schachtii* respectively)

BY

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Methods for the isolation of a hatching factor for the potato root eelworm (*Heterodera rostochiensis* Woll.) have been studied extensively by TODD and his associates and highly purified concentrates of this factor have been prepared (CARROLL, HEYES, JOHNSON & TODD, 1958). Experimental work has also been carried out by groups in the United States (MASSEY & NEAL, 1953; DAHLSTROM 1954) and in Holland (JANZEN & VAN DER TUIN, 1956) on this hatching factor. Although the existence of hatching factors for a number of closely related cyst-forming eelworm species has been demonstrated, no serious attempts have been made to purify and determine the nature of these substances. It is apparent, however, from the experiments of JONES & WINSLOW (1953) that different compounds must be involved in the hatching responses of the different eelworm species.

In the present paper a study has been made of hatching factors for the cabbage, hop and beet root eelworms, using methods that had earlier been applied to the potato root eelworm hatching factor. Root leachings from black mustard, hop and sugar beet were used as sources of the hatching factors of cabbage, hop and beet eelworms respectively while the mustard leachings also furnished a hatching factor for beet eelworm.

EXPERIMENTAL AND RESULTS

Bioassay of the hatching factors

The method of bioassay in these studies was similar to that used for the potato eelworm factor but some modifications were introduced. In Fenwick's method (FENWICK 1940; 1949), large batches of potato eel-

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worm cysts were separated from the soil and stored air-dry. Then for each assay, replicate batches of cysts measured out by number or by weight were presoaked in water for 7-10 days prior to introducing the extract under test. For the present experiments, the cysts were left in the soil until immediately before use. Then for each essay, weighed samples of air-dry soil were suspended in water and the material which floated was sieved through 20 and 60-mesh sieves; that retained on the 60-mesh sieve being filtered and the cysts recovered from the filter paper to form a replicate. The weight of soil was chosen so that each replicate contained approximately 100 cysts. No pre-soak was normally carried out. The counting of larvae was done as in the method of FENWICK (1952), but the results are given as number of larvae hatched per replicate rather than as L.A. (log.activity) values. The extracts were usually assayed at a series of different dilutions as in the potato eelworm studies.

Supply of starting material

Leachings for preliminary experiments were obtained from hop, sugar beet, and black mustard plants growing in pots of soil. Later work was concentrated on the mustard leachings and these were collected from black mustard grown in beds of soil and eventually from black mustard grown in beds of sand at the University of Reading. Experience in leaching these beds indicated that the potential supply of hatching factor was much greater than had been suspected in the earlier work with the potato eelworm hatching factor. Thus, when samples were taken at intervals during a continuous period of 10 hours leaching, the activity of the leachings seemed to be maintained throughout. This suggested that the hatching factor is excreted continuously by the plants but does not accumulate in the soil because it is destroyed by microorganisms or by other means. However, once removed from the soil in the form of leachings, the hatching factor appeared to be reasonably stable, particularly if the leachings were stored at low temperature.

Typical dose-response curves for the cabbage, hop and beet eelworms are shown in Fig. 1, Curve A. These are very similar to the curve obtained with the potato eelworm except that the hatch drops off much more rapidly in the case of the cabbage eelworm. Large numbers of larvae may hatch from cysts of the beet eelworm which are incubated in water alone, and this makes it more difficult to evaluate the results of assays of the hatching factor for this particular species.

Table I

Effect of heat, alkalinity, and butanol extraction at two pH values on the potency of three types of leachings as estimated by larval emergence from cysts incubated in the leachings and their extracts.

Each figure is the mean value for two samples of cysts.

	Larvae hatching per replicate in 14 days at Concentration Indicated.													
Treatment of leachings	Cabbage eelworm cysts in black mustard leachings				Hop eelworm cysts in hop leachings					Beet eelworm cysts in sugar beet leachings				
Concentration	1/4	1/8	1/16	1/32	1/4	1/12	1/36	1/108	1/324	1/4	1/12	1/36	1/108	1/324
Untreated leachings	1340	743	323	118	1330	1278	1053	325	83	2548	2060	1950	1750	768
Kept at 100° for 45 minutes	1503	1640	588	—	855	—	740	—	—	1503	—	—	—	—
Kept at pH 10 overnight	1368	1448	620	—	915	—	1260	—	—	1428	—	—	—	—
Distributed between n-butanol and water n-butanol soluble	40	—	—	—	1855	—	463	—	—	1238	—	—	—	—
water soluble	9	—	—	—	1463	—	783	—	—	2418	—	—	—	—
Distributed between n-butanol and dil. H_2SO_4 n-butanol soluble	355	—	—	—	1908	—	820	—	—	2518	—	—	—	—
water soluble	5	—	—	—	175	—	—	—	—	1615	—	—	—	—
Water controls		90					19					513 168 747		

Properties of cabbage, hop and beet eelworm hatching factors

The results of experiments performed on leachings from mustard, hop and sugar beet are summarized in Table I and in Figs. 1-3. The cabbage eelworm factor seemed to be quite stable to heat and to dilute alkali. The hop eelworm factor also was fairly stable, but there appeared to be partial inactivation of the beet eelworm factor (Table I).

In earlier studies where the potato eelworm factor was distributed between butanol and water, it was found mainly in the butanol phase at acid pH and mainly in the aqueous phase at neutral pH. This suggested that the active substance was an acidic compound, which in the acid form was more soluble in butanol and in the salt form was more soluble in water. Experiments with the hop and beet eelworm hatching factors suggested that they distribute themselves between butanol and water in a similar fashion (Table I). The cabbage eelworm hatching factor, however, appeared to be largely inactivated by this procedure and the little remaining activity was all in the butanol phase.

Since adsorption on charcoal and elution with aqueous acetone had been used for purification of the potato eelworm factor, this procedure was also applied to the hop, beet and mustard leachings (Fig. 1). Treatment with charcoal at the rate of 1 g/litre appeared to remove at least 90 % of the activity from each sample of leachings. The charcoal was recovered by siphoning off most of the supernatant and filtering the remainder with suction. It was then eluted by stirring with 4 lots of acetone: water (70 : 30). The cabbage eelworm factor was recovered in 25 % yield when a total of 12 ml/g of charcoal was used and in nearly 100 % yield when 60 ml/g were used. The hop eelworm factor was recovered in less than 1 % yield by using 10 ml/g of charcoal. The results for the beet eelworm factor were complicated by the variable behaviour and high water hatch of the eelworm in question but recovery seemed to be low when 10 ml/g of charcoal were used.

The results of these experiments indicated that the cabbage eelworm factor possessed certain advantages over the others for further investigation. It was recovered in good yield from the charcoal and it was also easier to assay because of the low water hatch and the steeper slope of the hatching curve (Figs. 1 and 2). Two-fold differences in concentration of this factor could be distinguished as readily as four- or five-fold differences of the other factors. Since leachings from black mustard contain a cabbage eelworm hatching factor and in ad-

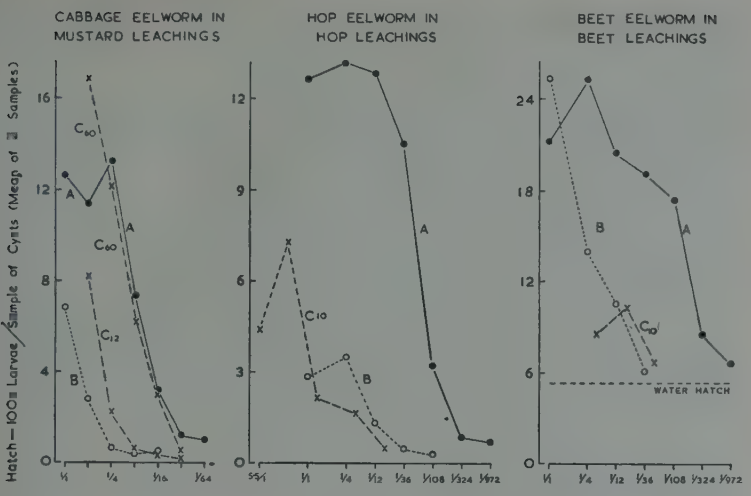

Fig. 1. Larval emergence from cysts of root eelworms incubated in root leachings from host plants growing in soil, and in extracts of these leachings. Curves: A—untreated leaching; B—aqueous residue of leachings after charcoal extraction; C_{10}, C_{12}, C_{60},—extract from charcoal eluted with 10, 12, or 60 ml of aqueous acetone/g charcoal.

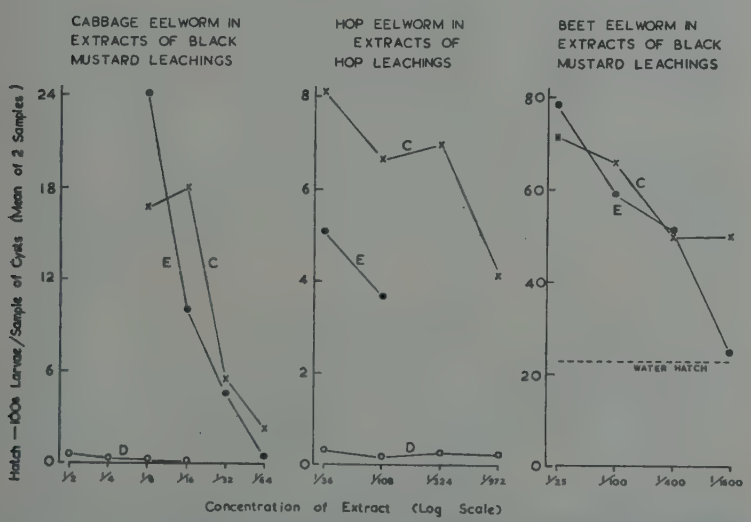

Fig. 2. Larval emergence from cysts of root eelworms incubated in extracts of root leachings from host plants grown in soil. Curves: C—charcoal eluate of leachings; D—charcoal eluate after extraction with phenol; E—phenol extract of charcoal eluate.

dition, a beet eelworm hatching factor, the production of this type of leachings was increased as the work progressed.

Some further methods which proved useful in the purification of the potato eelworm factor were also tested on a small scale with the cabbage, hop and beet eelworm factors. The first of these was the extraction of aqueous solutions of the active factors with phenol. Small samples of the material eluted from charcoal (equivalent to 10 litres of leachings in the cabbage eelworm experiment or to 2 litres in the hop and beet eelworm experiments) were made up in 10 ml of water and the solutions were extracted successively with 10 ml, 10 ml and 5 ml of water-saturated phenol. The combined phenol extracts were diluted with 100 ml ether and back-extracted successively with 15 ml, 10 ml and 10 ml of water. The combined aqueous extracts were washed with ether and concentrated to 10 ml for bioassay. As seen in Fig. 2, the cabbage and beet eelworm factors from black mustard leachings were readily extracted by phenol without much loss of activity. The hop

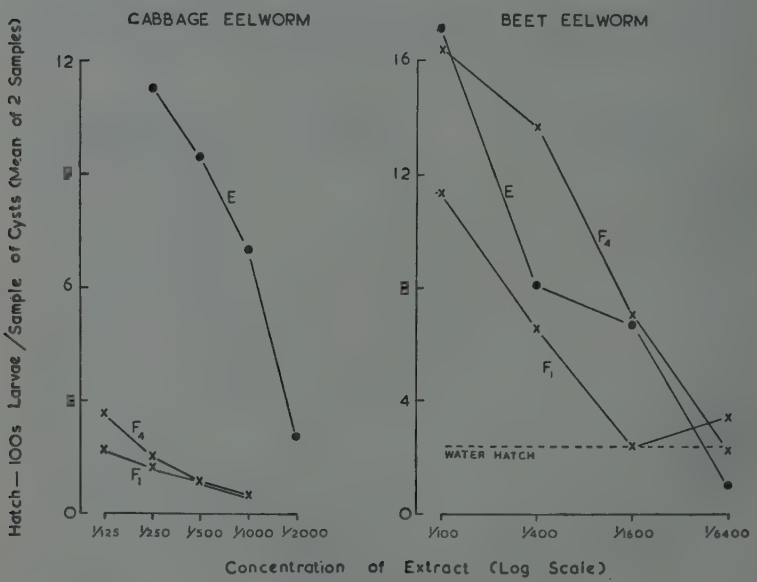

Fig. 3. Stability to formic acid treatment of the cabbage and beet eelworms hatching factors in a phenol extract of black mustard leachings. Curves: E—phenol extract of black mustard leachings: F_1, F_4,—phenol extract after treatment with 1N and 4N formic acid respectively.

eelworm factor was also extracted by phenol but there appeared to be some loss or inactivation.

Experiments on ion exchange chromatography with aqueous formic acid as the eluting solvent were also planned. Preliminary tests on the stability of the factors in mustard leachings to formic acid were carried out by mixing small quantities of the phenol extracts with 1N and 4N formic acid and concentrating the solutions to dryness as in the experiments with the potato eelworm hatching factor. The beet eelworm factor was stable towards this treatment but the cabbage eelworm factor was unfortunately destroyed (Fig. 3). This suggested that the cabbage and beet eelworm factors in black mustard leachings were different compounds. It also indicated that the ion exchange method which was very useful for purifying the potato eelworm factor, could not be used for the cabbage eelworm factor without modification. Some preliminary attempts were also made to purify the cabbage eelworm factor by counter-current extractions and by partition chromatography similar to that used in experiments on the potato hatching factor. These met with very little success and much of the activity was lost.

ACKNOWLEDGMENTS

The production of leachings and the bioassay of samples were conducted by Dr. R. D. Winslow through the co-operation of Mr. F. G. W. Jones at the School of Agriculture, University of Cambridge. The author is very appreciative of their contribution to the investigation. He is also indebted to Professor R. H. Stoughton, Department of Horticulture, University of Reading, for the production in bulk of black mustard leachings. He wishes to express his thanks to the Agricultural Research Council for financial support of this work and to Dr. A. W. Johnson and Professor Sir Alexander Todd for their interest and encouragement.

ZUSAMMENFASSUNG

1. Die Schlüpffaktoren für Kohl-, Hopfen- und Rübennematoden wurden unter Benutzung von Methoden untersucht, die früher beim Kartoffelnematoden angewendet wurden.

2. Die das Schlüpfen bewirkenden Faktoren wurden von Aktiv-Kohle leicht adsorbiert, aber nur der Schlüpffaktor des Kohlnematoden wurde in ausreichender Menge durch Auslaugen mit wässrigem Aceton wiedergewonnen. Ziemlich grosse Mengen des Lösungsmittels waren erforderlich.

3. Die Schlüpffaktoren konnten aus einer wässrigen Lösung durch Phenol extrahiert werden. Wenn sie durch Wasser und Butanol getrennt wurden verhielten sich die Hopfen- und Rübennematoden-Schlüpffaktoren wie der Kartof-

felnematoden-Schlüpffaktor, aber der Kohlnematoden-Schlüpffaktor wurde stark inaktiviert. Dieser wurde auch inaktiviert, wenn er einer wässrigen Ameisensäure-Lösung ausgesetzt wurde.

4. Die vorläufigen Ergebnisse weisen auf einige gemeinsame Eigenschaften der verschiedenen Schlüpffaktoren hin. Die Hopfen- und Rübennematoden-Schlüpffaktoren verhalten sich wie der Kartoffelnematoden-Schlüpffaktor, während der Kohlnematoden-Schlüpffaktor in einigen Punkten abweicht.

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Received for publication: January 6, 1958

Nematologica III (1958): 205-212. E. J. Brill, Leiden

THE ADULT FEMALE CUTICLE AND EGG SAC OF THE GENUS *MELOIDOGYNE* GOELDI, 1887

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The adult female cuticle or cyst wall of the genus *Heterodera* Schmidt, 1871 has been studied by several workers in recent years, both with regard to structure (Franklin 1939, Wieser 1953 and Ferris & Siegel 1957) and to chemical composition (Ellenby 1946 and Chitwood 1951). However, no work has been done on the female cuticle of the closely related genus *Meloidogyne* Goeldi, 1887 and very little on the egg sac of this animal.

Chitwood (1950) stated that the change of colour in the egg sac from colourless to orange during development possibly indicated tanning. He found that the egg sac was incompletely digested by pepsin and trypsin and gave positive xanthoproteic and ninhydrin reactions. He also found that when the exterior of the egg sac became orange in colour the contained eggs were more resistant to desiccation.

In this paper, both the chemical composition and structure of the adult female cuticle and egg sac of the genus *Meloidogyne* have been studied.

MATERIALS AND METHODS

Cuticle and egg sac material were obtained from specimens of *Meloidogyne hapla* Chitwood and *M. javanica* (Treub).

The cuticles for chemical analysis were obtained by puncturing females with needles and washing the cuticles free of internal contents and hypodermis by gentle pressure with needles in distilled water under the microscope. The egg sac material was similarly dissected away. Great care was taken to use pieces of material free from any adherent particles. This was especially difficult when freshly formed egg sac material was used because of its stickiness.

The material obtained for chemical analysis was used fresh and that required for sectioning was fixed in T.A.F. (Courtney, *et al.* 1955).

Sections

Frozen sections were cut 8 μ thick and examined in glycerine jelly. Wax sections were cut 2 μ thick and stained with Heidenhains azan. The iso-propanol method of De Guisti & Ezman (1955) was used for wax embedding.

Hydrolysis

Hydrolysis was carried out in sealed glass tubes at 105° C for twenty-four hours using 6N HCl. The HCl was evaporated off at 50° C and the hydrolysate was dissolved and stored in 10 % iso-propanol.

Paper Chromatography

Two-dimensional ascending paper chromatography was carried out at 17 ± 1° C in a glass chromotank using batches of Whatman No. 1 filter paper and 500 ml of solvent for each run. Papers were equilibrated overnight in the tanks before being lowered into the solvents. The first run was for six hours in n-butanol (77 % v/v), acetic acid (6 % v/v) and water (17 % v/v) (Hackman 1956) and the second for seven hours in saturated phenol/water + KCN. 80 % ethanol was also used as a solvent in unidimensional chromatography following elution of spots after a two-dimensional run. As previously described (Bird 1956) these solvents were removed from the paper and the ninhydrin and other more specific staining methods were used.

Histochemistry

Gautheret sitting drop slides (White 1954) sealed with molten petroleum jelly were used to examine the effect of enzyme hydrolysis and histochemical tests on the cuticle and egg sac. Pepsin and trypsin and the catechol, azo-coupling, Sudan Black and other reagents were used as previously described (Bird 1957).

RESULTS

Structure

1. The egg sac. Oil immersion studies of wax and frozen sections of egg sacs reveal that they consist of a sticky, structureless, clear, jelly-like substance. This substance is stained blue by the aniline blue involved in the Heidenhains azan technique, as is the cuticle. The stickiness of the egg sac appears to diminish as it becomes tanned and in this latter state pieces may be peeled off in layers. There is, however, no organized structure and it remains optically homogeneous.

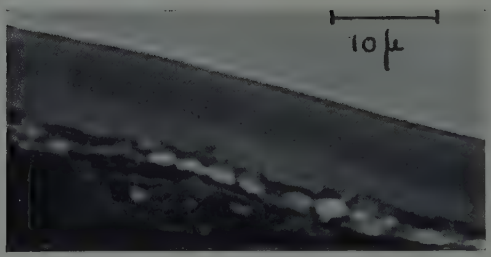

Fig. 1. Longitudinal section 2 μ thick, oil immersion of *Meloidogyne hapla* adult female cuticle stained with Heidenhains azan.

2. The structure of the female cuticle. There is considerable variation in the thickness of the female cuticle both in adults of different ages and in different regions of the same cuticle. The cuticle is thickest in the perineal region.

Both stained wax sections (Fig. 1) and unstained frozen sections examined under oil immersion showed similar structures, although the thinner wax sections gave more detail. These sections were obtained from fully grown females of *Meloidogyne hapla.*

The cuticle consists of a thin, darkly staining surface layer covering a homogeneous substance which is divided into three layers by two darkly staining bands of material which, in some cuticles, lie close to the hypodermis and give the impression of being a single darkly stained innermost layer. The thin, stained wax sections show vertical structures running from the hypodermis, to the outermost layer. These appear to be too regular to be caused by folds or sectioning artifacts and may be analogous with the channels in *Heterodera* mentioned by WIESER (1953).

Chemical Composition

1. Treatment with proteolytic enzymes. Pieces of egg sac and cuticle were placed in sitting drops of pepsin (B.D.H.) and trypsin (G. T. Gurr) solutions in Gautheret slides and incubated for twenty-four hours at 38° C. Pepsin brought about a partial hydrolysis of the cuticle and, to a lesser extent, the egg sac. Trypsin had a partial hydrolytic effect on the cuticle but did not appear to have any effect on the egg sac.

2. Paper chromatography. The hydrolysis products of the cuticle and egg sac were assessed qualitatively by paper chromatographic methods. They showed (Table I) some slight quantitative differences in that the egg sac contained more histidine, threonine and aspartic acid than the cuticle and less proline and arginine. The sulphur-containing amino acids appeared to be absent in the egg sac and no serine was observed in the cuticle.

As only very small quantities of material were used in these experiments due to the time and difficulties involved in obtaining clean samples, it is possible that other amino acids might be present in concentrations below those necessary for a positive ninhydrin reaction.

The presence of arginine, histidine and proline was verified by specific tests as previously described (BIRD, 1956). Elution of cut-outs from the leucine/iso-leucine area after two-dimensional chromatography followed by a uni-dimensional run in 80% ethanol has shown the

TABLE I

Amino acids demonstrated by paper chromatography of acid hydrolysates of egg sac and female cuticle of the genus Meloidogyne

Egg Sac		*Female Cuticle*
glycine	—	glycine
alanine	—	alanine
serine	—	———
threonine	>	threonine
———	—	cystine
———	—	cysteic acid
valine	—	valine
leucine / iso-leucine	—	leucine / iso-leucine
aspartic acid	>	aspartic acid
glutamic acid	—	glutamic acid
arginine	<	arginine
lysine	—	lysine
tyrosine	— very slight —	tyrosine
histidine	>	histidine
proline	<	proline
hydroxy-proline	— very slight —	hydroxy-proline

presence, in both the egg sac and cuticle, of an unknown spot with an R_f value, in this solvent, of 0.18, in addition to these amino acids. This does not compare with the R_f value of phenyl alanine, the only other common amino acid which occurs in this area in the prepared map of known amino acids. Similarly, elution of the aspartic acid area followed by a uni-dimensional run in 80 % ethanol has shown, in addition to aspartic acid, an unknown spot with an R_f value of 0.40.

Tests for tryptophane in the egg sac and cuticle were negative. Thus (Table I) at least fourteen amino acids have been identified as components of the egg sac and at least fifteen amino acids have been identified as components of the cuticle.

3. Treatment with reagents. The cuticle and egg sac were treated with various reagents in an attempt to determine the types of forces involved in stabilizing their structure. These experiments were mostly carried out at room temperature in either welled or sitting drop slides sealed with molten petroleum jelly.

The following reagents had no noticeable effect on either the cuticle or egg sac after twenty-four hours treatment: 0.2N HCl, 0.2N NaOH, 6M urea, formamide, 2M $CaCl_2$ and saturated calcium thiocyanate. Those reagents which produced an effect on either the egg sac or the cuticle or both and the results of histochemical investigation have been tabulated (Table II).

Boiling distilled water (sealed tube at 105° C for twenty-four hours),

TABLE II

Results of the reactions of the egg sac and cuticle of the genus Meloidogyne *to various reagents*

Reagents	*Egg Sac*	*Female Cuticle*
boiling distilled water (24 hours)	dissolved	outer layer remained
boiling 0.2N NaOH (5 min.)	dissolved	dissolved
0.5M thioglycollate	undissolved	outer layer remained
10% sodium hypochlorite	dissolved	outer layer remained
Molisch reaction	positive	negative
0.1% catechol at 40° C for 1½ hours	tanned	outer layer tanned
0.1% catechol + KCN at 40° C for 1½ hours	tanning inhibited	tanning inhibited
0.1% catechol + sodium diethyldithiocarbamate at 40° C for 1½ hours	tanning inhibited	tanning inhibited
azocoupling reaction	positive	outer layer positive
Sudan III in colloidal suspension	negative	outer layer faintly positive
Sudan Black B	negative	outer layer faintly positive

0.5M thioglycollate and 10% sodium hypochlorite all partially broke down the cuticle. In each case, only the thin, outermost layer remained. The egg sac was not affected by 0.5M thioglycollate but it was completely broken down by boiling water and 10% sodium hypochlorite. Both the egg sac and cuticle were completely broken down by boiling for a short time in 0.2N NaOH. The egg sac gave an intense positive Molisch reaction indicating the presence of carbohydrate which had been suspected as considerable humin formation was observed during acid hydrolysis of the egg sac while none occurred during the hydrolysis of the cuticle which gave a negative Molisch reaction. Treatment with 0.1% catechol in distilled water at 40° C for 1½ hours (JOHRI & SMYTH, 1956) in sitting drop slides induced tanning both in the egg sac and the outermost layer of the cuticle. This reaction was inhibited both by KCN and sodium diethyldithio-carbamate which is strong evidence for the presence of the copper-containing enzyme polyphenol oxidase. In addition, the azo-coupling reaction (GOMORI, 1952) for phenolic substances (using fast red salt B-E Gurr Ltd) was positive in the egg sac and the outermost layer of the cuticle in frozen sections. After treatment with hypochlorite followed by washes in chloroform and acetone and re-treatment with hypochlorite, the thin, outermost layer was broken down but not completely dissolved. This suggests a

lipoprotein complex. Treatment with Sudan III in colloidal suspension and Sudan Black B (Pearse, 1953) gave faintly positive reactions for the cuticle but were negative for the egg sac.

These results indicate that the egg sac of the genus *Meloidogyne* is a tanned glycoprotein of uniform composition. The adult female cuticle consists of a thin tanned lipoprotein layer covering a thicker less resistant homogeneous layer which is split by two bands of more darkly staining tissue.

DISCUSSION

The cuticle of the genus *Meloidogyne* differs markedly from that of the genus *Heterodera* in that frozen sections show that it is not divided into two obvious parts, the exo- and endo-cuticle. These are clearly shown in Wieser's (1953) photographs which frozen sections of *Heterodera schachtii* cut in this laboratory substantiate. *Meloidogyne* cuticle bears a structural resemblance to the endo-cuticular portion of *Heterodera* cuticle.

At our present state of knowledge of the structure of the cuticles of nematodes in general, it does not seem to be wise to attempt to postulate that the presence of an exo- and endo-cuticle is, perhaps, common to all nematodes, as has been done by Wieser; neither is it wise to compare the highly specialized cyst wall of *Heterodera* with the cuticle of *Ascaris lumbricoides*. However, attempts such as these to find the common basic pattern of cuticular structure should be of considerable benefit when more is known about these structures in a wider range of nematodes.

As a result of the above experiments on the chemical composition of the egg sac and cuticle, it seems probable that both these structures resemble the cuticles of other nematodes which have been studied (Bird & Rogers, 1956; Bird, 1957) in that they resemble secreted collagens in their reactions to various reagents. Furthermore, both the egg sac and, to a lesser extent, the cuticle contain phenolic compounds and the enzyme polyphenol oxidase. The visible change in colour of the egg sac from colourless to orange-brown is thus almost certainly a tanning reaction similar to that described by Ellenby (1946) in *Heterodera rostochiensis*.

Differences are that the egg sac is more heavily tanned and contains more carbohydrate than most other cuticles studied and that the *Meloidogyne* cuticle appears to have a tanned lipoprotein layer rather than a thin lipid layer overlying a tanned layer as in *Ascaris lumbricoides*.

This thin tanned lipoprotein layer in *Meloidogyne* is very fragile and difficult to work with.

Little is known about the origin and secretion of the egg sac. Whilst the cuticle of nematodes is probably derived from the hypodermis or subcuticle, the egg sac of the genus *Meloidogyne* is considered by Chitwood (1950) to belong to a group of compounds which, in some nematodes, e.g. *Ascaris*, are found as an outer protein coat covering their eggs. The egg sac would, under these circumstances, be a uterine secretion. Nagakura (1930) is also of this opinion.

I wish to thank Mr F. Penman for his advice and criticism, Mr L. Smith for technical assistance, Mr E. Lawton for the photography involved and Mr J. Fisher of the Waite Agricultural Research Institute for providing specimens of *Heterodera schachtii*.

ZUSAMMENFASSUNG

Der Eiersack der Gattung *Meloidogyne* besteht in seinem frühen Stadium aus einem strukturlosen, klaren, gelatineähnlichen Glycoprotein, das durch die Wirkung von Polyphenoloxydase auf Phenolbestandteile im Eiersack ausgefällt wird. Dieses Protein besteht aus wenigstens 14 Aminosäuren.

Die Kutikula des erwachsenen Weibchens der Gattung *Meloidogyne* scheint aus einer dünnen, koagulierten Lipoproteinoberflächenschicht zu bestehen, die eine homogene Substanz bedeckt, welche durch zwei dunkelgefärbte Bänder in drei Schichten getrennt ist. Das Protein der Kutikula besteht als wenigstens 15 Aminosäuren.

Auf Grund ihres Verhaltens gegenüber verschiedenen Reagentien können Eiersack und Kutikula als ein abgesondertes Kollagen bezeichnet werden.

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Received for publication: January 28, 1958

Nematologica III (1958): 213-228. E. J. Brill, Leiden

NEBENWIRKUNGEN BEI DER NEMATODENBEKÄMPFUNG MIT SHELL D-D UND VERWANDTEN MITTELN

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Shell D-D [1]) und andere Dichlorpropan-Basis aufgebaute Präparate haben sich als geeignete Mittel zur Bekämpfung pflanzenparasitischer Nematoden erwiesen. Neben einer nematiziden Wirkung besitzen sie auch andere Eigenschaften. So ist bekannt, dass sie phytotoxisch sind und bei einer Bodenbehandlung im Frühjahr mitunter eine Geschmacksbeeinflussung nachfolgender Kulturen, z.B. von Möhren und Kartoffeln verursachen (GOFFART, 1954). Neben diesen Nachteilen sind auch einige Vorteile zu verzeichnen. Eine der wichtigsten Erscheinungen ist der wachstumsfördernde Einfluss, den eine DD-Behandlung auf die unmittelbar folgende und zuweilen auch noch auf die zweite Nachfrucht ausüben kann. Über dieses Problem berichteten bereits CARTER (1943), später auch PETERS (1948), EDWARDS (1950) und GRAINGER (1954). Die Autoren sprechen meist von einem „Soil amendment effect" oder von einer „Stimulation", ohne indes die Wirkung zu präzisieren. Auch auf der Pflanzenschutztagung in Bad Neuenahr (1954) wurde über das Problem der Nebenwirkung von DD diskutiert (S. 127/128). Positiven Beobachtungen mit einer deutlichen Wachstumsförderung standen negative Befunde gegenüber. OOSTENBRINK (1955) schliesst sich hier der Ansicht STEINERS (1947) an und meint, dass der „stimulierende" Einfluss des DD auf die abtötende Wirkung von Nematoden zurückzuführen ist, die man bislang noch nicht gefunden hat. Diese Erklärung mag für manche Fälle zutreffen, kann aber nicht das Problem restlos klären, wie GRAINGER (1954) betont. Im Jahre 1957 bot sich uns mehrfach Gelegenheit, eine solche Wachstumsförderung mit DD und ähnlichen Präparaten bei Zucker- und Futterrüben zu beobachten. Über einen konkreten Fall, der näher untersucht wurde, soll nachstehend berichtet werden.

Auf einer Fläche, auf der am 20. Oktober 1955 u.a. Bekämpfungs-

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1) Im weiteren Verlauf kurz als DD bezeichnet.

versuche mit DD gegen Kartoffelnematoden (*Heterodera rostochiensis* Wollenweber) durchgeführt worden waren und die 1956 Kartoffeln getragen hatte, zeigten sich 1957 an Futterrüben auffällige Wachstumsdifferenzen. Die Wuchsunterschiede, die schon kurz nach dem Auflaufen der Saat hervortraten und bis zur Ernte deutlich erkennbar blieben, fielen genau mit den Grenzen der 1955 mit 40 und 80 ml/qm DD behandelten Parzellen zusammen. Hier war die Rüben- und Blattentwicklung der Pflanzen wesentlich besser als auf der unbehandelten Fläche. Während der ganzen Vegetationsperiode konnte immer wieder beobachtet werden, dass die Rüben auf den behandelten Flächen an heissen Tagen weniger rasch und vollständig welkten und ihre Vollturgeszenz nach solchen Perioden schneller wieder erreichten als die Pflanzen der unbehandelten Teilstücke. Schäden durch Rübenfliege (*Pegomyia hyoscyami*) und Saugschäden durch die Schwarze Bohnenlaus (*Doralis fabae*) traten nicht auf, da die Rüben zweimal mit Metasystox (800 ml/ha) behandelt worden waren. Infolge dieser Massnahme war auch der Vergilbungsbefall trotz stärkster Virusverseuchung unbehandelter Rübenschläge in der Umgebung relativ schwach. Eine Feststellung der Parzellenerträge war aus technischen Gründen nicht möglich; daher wurden die Blatt- und Rübengewichte von jeweils 10 sorgfältig ausgewählten Durchschnittspflanzen der behandelten und unbehandelten Teilstücke bestimmt. Dabei ergaben sich für die unbehandelten Parzellen mittlere Blatt- und Rübengewichte von 170 und 700 g gegenüber 210 und 940 g für die DD-Parzelle. Bei der Unsicherheit, die Ertragsfeststellungen an Einzelpflanzen anhaftet, können die Gewichtsunterschiede nur als Hinweise auf die Grössenordnung der Wuchsdifferenzen gewertet werden; bemerkenswert erscheint jedoch, dass die beiden Pflanzengruppen in der Entwicklung des Rübenkörpers stärker voneinander abweichen als in der Entwicklung des Blattsystems (Relativer Mehrertrag der DD Pflanzen: Blatt 123,5, Rübe 134,3, wenn Unbehandelt = 100 gesetzt. Durch vergleichende Boden- und Pflanzenuntersuchungen wurden weder Rübennematoden (*Heterodera schachtii*) noch andere an Rüben schädigend auftretende Nematoden nachgewiesen. Namentlich wurden auch keine wandernden Nematoden der Gattungen *Pratylenchus, Paratylenchus* und *Tylenchorhynchus* festgestellt, obwohl von jeder Parzelle 3 × 100 g der an den Rübenwurzeln haftenden Erde untersucht wurden. Die nematizide Eigenschaft des Mittels konnte daher nicht Ursache der Wuchsunterschiede sein; vielmehr musste als solche eine unmittelbare oder mittelbare Wirkung auf das Wachstum der Rüben angenommen werden.

Da die Unterschiede der beiden Pflanzengruppen im Welkeverhalten auf eine Beeinflussung des Wasserhaushalts durch das nematizide Mittel hindeuten, erfolgten zunächst Untersuchungen einiger wichtiger Wasserhaushaltsfaktoren am Standort und dann weitere Bestimmungen an entnommenen Pflanzen im Laboratorium.

Das Blattmaterial für beide Untersuchungsserien wurde am 5. Sept. 1957 gegen 15 Uhr entnommen. Für die Bestimmungen kamen nur völlig intakte, grüne Blätter, die keinerlei Beschädigungen, insbesondere keine Virusbefallssymptome, zeigten, in Betracht. Bei der Entnahme befanden sich die Pflanzen, soweit äusserlich erkennbar war, in vollturgeszenten Zustand. Die Temperatur betrug im Zeitpunkt der Untersuchungen 17° C bei einem Tagesmaximum von 19° C; die Witterung war sonnig. Am Nachmittag des 4. Septembers und in der Nacht vom 4. zum 5. September waren insgesamt 13,2 mm Niederschlag gefallen, in der Pentade vor dem Untersuchungstermin insgesamt 27,2 mm. Durch die Höhe dieser Niederschlagsmengen war auch auf dem leichten, humosen Sandboden des Versuchsfeldes eine hinreichende Wasserversorgung des Pflanzenbestandes zu dieser Zeit gewährleistet, zumal in den Vormonaten reichliche, z.T. überdurchschnittliche Regenmengen gefallen waren (August: 85,1 mm, Juli: 114 mm). Die Monate April, Mai und Juni waren dagegen durch mehr oder weniger länger anhaltende Dürreperioden und geringe Niederschlagsmengen charakterisiert (April: 13,0 mm, Mai: 15,4 mm, Juni: 37,7 mm).

ERGEBNISSE

1. Bestimmungen am Standort

Zur Feststellung des aktuellen Blattwassergehaltes, der Oberflächenentwicklung, des Sukkulenzgrades und des Wasserdefizits wurden mit einem scharfrandigen Bohrzylinder kreisrunde Blattstückchen aus dem unteren Drittel völlig normaler, mittelgrosser Blätter beider Pflanzengruppen so ausgestanzt, dass die 6,15 cm^2 grossen Stücke keine grösseren Blattnerven enthielten. Etwa 20 dieser Blattstücke je Gruppe wurden in vorgewogene Trockenschälchen mit eingeschliffenen Deckeln gegeben und sorgfältig vor Verdunstung geschützt, nach der Rückkehr im Laboratorium ohne Verzug auf einer analytischen Waage (Empfindlichkeit 0,1 mg) gewogen sowie dann im elektrischen Thermostaten bei 105° C bis zur Gewichtskonstanz getrocknet. Die Blattproben zur Bestimmung des osmotischen Wertes, die ebenfalls jeweils mittelgrossen Blättern entstammten und keine Anteile an Hauptblattnerven bezw. Blattstielen enthielten, wurden in den üblichen kryo-

skopischen Röhrchen gesammelt, sofort luftdicht mit Korkstopfen verschlossen und in Metallzylinder mit Schraubdeckelverschluss gegeben. Nach halbstündigem Erhitzen im kochendem Wasserbad wurden die Proben bis zur Untersuchung im Eisschrank bei —1° C aufbewahrt. Die kryoskopische Untersuchung erfolgte nach den Angaben von WALTER unter Verwendung eines Gefrierthermometers nach *Burian-Drucker* mit 1/50° Einteilung. Die Feststellung des Blattwassergehaltes bei voller Sättigung fand an Blättern der gleichen Pflanzen statt, denen die übrigen Proben entnommen waren. Diese Blätter wurden nach Kürzung der Blattstiele unter Wasser mittels eines scharfen Rasiermessers in Leitungswasser an schattiger Stelle unter Glasglocken in wasserdampfgesättigter Atmosphäre etwa 24 Stunden zur Aufsättigung belassen. Die Ergebnisse dieser Untersuchungen sind in Tabelle I wiedergegeben.

In allen Zustandswerten weisen die Pflanzen beider Gruppen charakteristische Unterschiede auf. Die Rübenblätter der 1955 mit DD behandelten Flächen zeigen einen höheren aktuellen Wassergehalt, niedrigere aktuelle osmotische Werte und ein geringeres Wassersättigungsdefizit, das allerdings auch bei den Kontrollpflanzen nicht sehr

TABELLE I

Ergebnisse der Untersuchungen am Standort

	Unbehandelte Fläche	Behandelte Fläche
Blatt-Wassergehalt in % am Standort	82,82	83,26
Blatt-Wassergehalt in % bei voller Sättigung	85,67	85,70
Wassersättigungsdefizit bei Entnahme in %	3,32	2,85
Sukkulenzgrad, g H_2O/dm^2 [1])	2,85	3,38
Oberflächenentwicklung, dm^2/g Blattfrischgewicht [1])	3,01	2,54
Osmotischer Wert in atm, am Standort	13,12	12,40
Osmotischer Wert in atm, bei voller Sättigung	10,72	9,39

hoch liegt. Die Blätter wiesen ausserdem eine eindeutig geringere Oberflächenentwicklung und gleichzeitig einen höheren Sukkulenzgrad auf. Bei voller Sättigung war im Wassergehalt zwischen den beiden Blattgruppen kein Unterschied mehr festzustellen, während der osmotische Druck der mit DD behandelten Pflanzen noch merklich unter dem der unbehandelten lag.

Alle Werte sind Zeichen einer besser ausgeglichenen Wasserbilanz und einer geringeren Welkeanfälligkeit der nach einer DD-Behandlung angebauten Rüben.

1) Bei dieser Berechnung wurde die Grösse der einfachen Blattfläche zugrunde gelegt.

2. Laboratoriumsversuche

Die Untersuchung des Welkeverhaltens der beiden Pflanzengruppen erfolgte a) durch Bestimmung der Veränderung von Wassergehalts- und osmotischen Werten allmählich austrocknender Blätter bei ziemlich langen Zeitinterwallen der Einzelmessungen, b) durch unmittelbare kurzfristige Feststellungen des Transpirationsverlaufs welkender Blätter.

Zu a) Gleichzeitig mit den oben beschriebenen Untersuchungen am Standort wurden von jeder der beiden Pflanzengruppen 50 gut ausgebildete mittlere Blätter entnommen, in dicht schliessende Kästen verpackt und sofort nach Ankunft im Laboratorium (etwa 30 Minuten nach Entnahme) an einem schattigen Ort zum Welken mittels kleiner Stahldrahthaken aufgehangen. In gewissen Zeitabständen erfolgten dann an jeweils 10-15 Blätter je Gruppe Bestimmungen von Wassergehalt und osmotischem Wert des Pressaftes. Die hierzu nicht benötigten Blätter wurden nach Kürzung des Blattstiels unter Wasser zur Aufsättigung in Gefässe mit Leitungswasser gestellt und Verlauf und Schnelligkeit des Wiederturgeszentwerdens beobachtet. Nach Beendigung des Wiederaufsättigungsvorganges erfolgte erneut eine Bestimmung von Wassergehalt und osmotischem Wert der Blattproben.

Aus den Werten der Tabelle II geht eindeutig hervor, dass der Welkevorgang bei den Pflanzen der mit DD behandelten Parzelle wesentlich langsamer verläuft als bei den Kontrollpflanzen; erst nach 120 Stunden Welkedauer zeigen Blatt-Wassergehalt und osmotischer Wert des Pressaftes etwa gleich hohe Werte. Dementsprechend liegen

Tabelle II

Veränderung von Wassergehalt und osmotischem Wert langsam welkender Rübenblätter nach Behandlung mit DD

	Wassergehalt in % des Frischgewichtes		Osmotischer Wert	
	Unbehandelt	Behandelt	Unbehandelt	Behandelt
bei Untersuchung am Standort	82,82	83,26	13,12	12,40
nach voller Sättigung	85,67	85,70	10,72	9,39
nach 24 Std. Welkedauer	79,80	81.20	17,32	13,84
„ 96 „ „	73,70	74,05	24,99	23,56
„ 120 „ „	72,68	72,00	25,95	25,95

auch die in den ersten Welkephasen erreichten Wasserdefizite bei den DD-Pflanzen niedriger als bei den Kontrollen (Tab. III).

Das Verhalten der beiden Blattgruppen bei der Wiederaufsättigung

geht aus den Angaben der Tabellen IV und V hervor. Bei den Rübenblättern der DD-Parzellen ist demnach nicht nur der Welkevorgang langsamer verlaufen, sondern auch die Erholung nach Wiederbeginn der Wasserzufuhr schneller und in höherem Grade eingetreten. Nach nur 24-stündigem Welken haben alle Blätter beider Pflanzengruppen

Tabelle III

Wassersättigungsdefizit in %

	Unbehandelt	Behandelt
Im Felde	3,32	2,85
nach einer Welkezeit von 24 Std.	6,80	5,20
„ „ „ „ 96 „	14,00	13,55
„ „ „ „ 120 „	15,20	16,00

ihre volle Turgeszenz wieder erlangt, wie die entsprechenden Wassergehaltswerte anzeigen; nach 96 und 120 Stunden Welkezeit ist ein Teil der Vergleichsblätter nicht mehr turgeszent geworden, während von

Tabelle IV

Wiederaufsättigung gewelkter Rübenblätter von DD-behandeltem und unbehandeltem Boden

	von unbehandeltem Boden			von DD-behandeltem Boden		
	volltur-geszent	teiltur-geszent	welk	volltur-geszent	teiltur-geszent	welk
nach 24 Std. Welkedauer	10	0	0	10	0	0
„ 96 „ „	4	4	2	5	5	0
„ 120 „ „	0	5	5	4	6	0

Als teilturgeszent wurden solche Blätter bezeichnet, bei denen mehr oder weniger grosse Blatteile eingetrocknet waren, während der übrige Teil der Spreite (mindestens 50 %) und der Blattstiel wieder straff geworden waren. Bei den als welk bezeichneten Blättern war eine Wasseraufnahme nicht mehr eingetreten.

den Blättern der DD-Parzelle klein Blatt völlig verwelkt ist. Die Unterschiede im Erholungsvermögen nach der Welkeperiode zeigen sich auch in den Wassergehaltszahlen und noch deutlicher in den osmotischen Werten des Blattpressaftes der wiederaufgesättigten Blätter beider Pflanzengruppen (Tab. V). Die nicht volle Kongruenz dieser beiden Faktoren hat vermutlich darin ihren Grund, dass vor der Wassergehaltsbestimmung die welk gebliebenen Blätter bezw. Blatteile ausgeschieden wurden, während die kryoskopischen Proben dem Durchschnitt aller Versuchsblätter entsprechen. Der Vergleich der Werte in Tabelle V zeigt jedenfalls deutlich, dass nach längeren Welkeperioden

Tabelle V

Wassergehalt und osmotischer Wert nach Wiederaufsättigung

	Blattwassergehalt in % des Frischgewichtes		Osmotischer Wert des Pressaftes in atm.	
	Unbehandelt	Behandelt	Unbehandelt	Behandelt
Bei voller Sättigung	85,67	85,70	10,72	9,39
Nach 24 Std. Welkedauer	85,85	86,53	10,72	9,17
„ 96 „ „	80,25	85,17	17,80	14,92
„ 120 „ „	83,14	85,57	19,00	15,64

die Blätter der DD-Parzelle in weitaus höherem Grade sich ihrem Normalzustand wieder angenähert haben als die Vergleichspflanzen.

Zu b) Zur unmittelbaren Bestimmung des Transpirationsverhaltens der beiden Blattgruppen wurden aus vollturgeszenten Blättern kreisrunde Blattstücke (20,42 cm^2) ausgestanzt, spätestens 2 Minuten nach dem Ausstanzen auf einer Balken-Torsionswaage (Empfindlichkeit 1 mg) gewogen, neben der Waage am Arbeitsplatz im Laboratorium in diffusem Licht (Nordfenster) aufgehangen und mehrfach nach jeweils genau 20 Minuten erneut gewogen. Die Raumtemperatur betrug am Versuchstage 18° C. Unter den gegebenen Versuchsbedingungen kann nach den Ergebnissen von Vorversuchen mit variierten Wägungsintervallen die Wasserabgabe der Blattstücke in der ersten Wägungsperiode mit grosser Annäherung als aktuelle Transpiration angesehen werden, bei der die kutikulare Komponente jedoch quantitativ überwiegen dürfte, da auf abgeschnittenen Rübenblättern auch bei ungehinderter Wasserzufuhr die Mehrzahl der Stomata meist weitgehend geschlossen ist. Aus dem in Abb. 1 wiedergegebenen Verlauf der Wasserabgabe während der insgesamt zweistündigen Versuchsdauer ergibt sich, dass 1. bei voller Turgeszenz die Wasserabgabe der von DD beeinflussten Blätter deutlich niedriger ist als der Wasserverlust der Vergleichsblätter, dass jedoch 2. der auf zunehmender Austrocknung beruhende Transpirationsabfall in den folgenden Wägungsperioden bei den Kontrollpflanzen steiler ist als bei den Pflanzen der DD-Parzelle, sodass sich in den späteren Perioden die Werte zwar stärker angleichen, die Unterschiede jedoch nicht völlig verschwinden.

Nachdem die regelmässigen Messungen in 20 Minuten-Perioden nach 2 Stunden Versuchsdauer eingestellt worden waren, wurde genau 5 Stunden nach Versuchsbeginn noch eine letzte Wägungsserie durchgeführt. Die in diesen 3 Stunden abgegebenen Wassermengen waren bei den Pflanzen der DD-Parzellen, trotzdem der Endpunkt des Welkens zu diesem Zeitpunkt bereits mit grosser Annäherung erreicht war,

immer noch etwas niedriger als die Wasserverluste der Kontrolle. Die Unterschiede in der Transpirationsintensität waren bei Verwendung des Blatt-Frischgewichts als Bezugssystem noch grösser als bei Berechnung auf die Blattfläche (Tab. VI).

Das Ergebnis dieser Untersuchungen stimmt also gut mit dem der

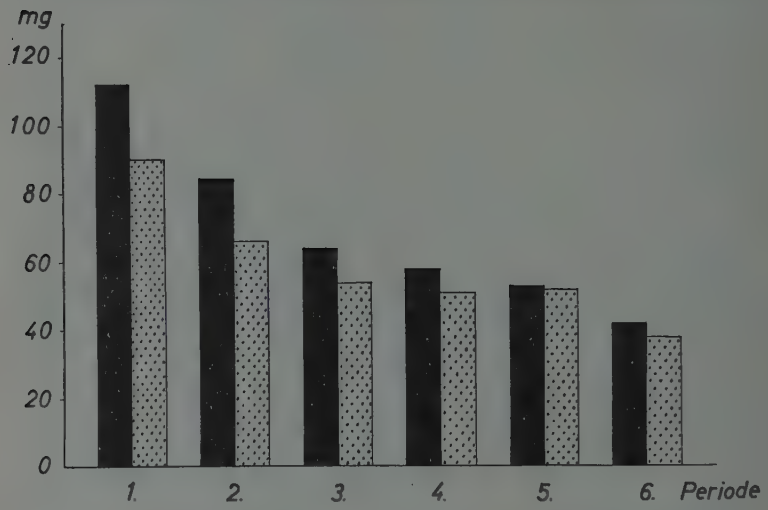

Abb. 1. Wasserabgabe von Blattstücken in mg/dm² in aufeinanderfolgenden Wägeperioden von je 20 Minuten Dauer.
Schwarz: Unbehandelt.
Punktiert: Behandelt.

Tabelle VI

Wasserabgabe der Rübenblätter von DD-behandelten und unbehandelten Parzellen

	Unbehandelt	Behandelt absolut	Diff.
Welke-Transp., mg/dm² in 20 Min.	109,7	88,2	—19,6 %
„ „ , mg/g Frischgew. in 20 Min.	33,4	23,8	—28,7 %
„ „ , mg/dm² Frischgew in 2 Std.	404,2	343,8	—15,0 %
„ „ , mg/g Frischgew. in 2 Std.	123,8	92,7	—25,1 %
„ „ , mg/dm² in den folgenden 3 Std.	317,2	309,2	— 2,5 %
„ „ , mg/g Frischgew. in den folgenden 3 Std.	110,1	92,0	—17,4 %
„ „ , mg/dm² in 5 Std. (Ges. Versuchsdauer)	721,4	653,0	— 9,5 %
„ „ , mg/g Frischgew. (Ges. Versuchsdauer)	219,6	176,3	—19,7 %

Versuche überein, in denen Wassergehalt, osmotischer Wert und Wiederaufsättigungsvermögen austrocknender Rübenblätter untersucht wurden. Die Wasserabgabe der von DD beeinflussten Rübenblätter ist unter gleichen Bedingungen weniger intensiv als die der unbeeinflussten Kontrollpflanzen, d.h. die erstgenannten Blätter welken langsamer und erreichen schon deshalb die für den Übergang vorübergehenden Welkens zum irreversiblen Welken kritischen Punkte, nämlich letales Wassersättigungsdefizit und „O Max" (maximaler osmotischer Druck) im Sinne Walters weniger rasch als die Vergleichspflanzen. Zudem liegen diese kritischen Werte bei den DD-Blättern noch höher als bei den Kontrollen. Die Pflanzen der DD-Parzellen welkten demnach nicht nur langsamer, sondern ertrugen auch einen höheren Grad der Austrocknung, besassen also eine erheblich gesteigerte Welkeresistenz.

3. Untersuchungen des Kohlehydrat- und Stickstoffstoffwechsels der Rüben von DD- und unbehandelten Parzellen

An jeweils 10 Pflanzen der beiden Pflanzengruppen wurden der Gehalt an Trockensubstanz, reduzierendem und hydrolysierbaren Zucker, löslichem und nicht löslichem Stickstoff in Blattspreite, Blattstiel und Rübe nach der am Institut üblichen Methode (Steudel Heiling, 1954) durchgeführt (Tab. VII).

Tabelle VII

Chemische Untersuchungen an den Rüben der mit DD behandelten und unbehandelten Parzellen

	Blattspreite		Blattstiel		Rübe	
	Unbehandelt	Behandelt	Unbehandelt	Behandelt	Unbehandelt	Behandelt
Trockensubstanz in %	14,17	13,98	9,80	9,34	12,64	11,15
Asche in % der Trockensubstanz	14,33	17,07	—	—	—	—
Gesamtzucker mg/g Frischgewicht	21,27	27,70	39,85	32,40	85,08	75,6
Löslicher Stickstoff mg/g Frischgew.	1,46	1,16	0.52	0,57	0.87	0.98
Nichtlöslicher Stickstoff mg/g Frischgew.	4,88	4,53	1,27	1,25	0,77	0,74

Bei diesen Untersuchungen haben sich keine signifikanten Unterschiede im Kohlehydrat- und Stickstoffstoffwechsel zwischen den beiden Pflanzengruppen ergeben. Geringfügige Differenzen bei einzelnen Faktoren liegen entweder überhaupt innerhalb der normalen Varia-

bilität oder stehen in unmittelbarem oder mittelbarem Zusammenhang mit der ungleichen Wasserversorgung, die sich aus den Wassergehaltswerten ergibt. Diese liegen für Blattspreite, Blattstiel und Rübe bei den Pflanzen der DD-Parzellen eindeutig höher. Das wesentlichste Ergebnis ist aber wohl die Feststellung, dass der Gehalt an löslichem und nicht löslichem Stickstoff in Spreite, Blattstiel und Rübe bei beiden Pflanzengruppen im wesentlichen gleich war. Da diese Faktoren bei der Rübe äusserst variabel sind und insbesondere auf Unterschiede in der Stickstoffversorgung des Standortes mit grossen Ausschlägen reagieren, ist ein höherer Stickstoff-Vorrat auf den DD-Parzellen als Ursache des besseren Wachstums nicht anzunehmen. Ein eindeutiger Unterschied zwischen den Pflanzen der DD-Parzellen und denjenigen der Vergleichsflächen ergab sich lediglich im Aschengehalt der Blattspreite, der bei den DD-Pflanzen erheblich höher lag als bei den Kontrollen.

4. Untersuchungen über den unmittelbaren Einfluss einer DD-Behandlung auf den Boden

Im Oktober 1957 wurde auf einem benachbarten Feldstück ein neuer Bekämpfungsversuch mit DD (60 ml/qm) angelegt. Von diesen Parzellen und den unbehandelten Vergleichsflächen wurden am 22.11.57 Bodenproben entnommen und in Zellophanbeutel im Laboratorium aufbewahrt. Aus technischen Gründen konnte mit der Untersuchung der Proben erst am 28.12.57 begonnen werden. Sie wurden auf Wassergehalt, Geschwindigkeit der Austrocknung und ihren Elektrolyt-Gehalt durch Bestimmung der Leitfähigkeit wässriger Bodenextrakte untersucht (100 g Frischerde wurde in 250 ml Wasser ausgeschüttelt und nach 48 Stunden filtriert) [1]). Zur Ermittlung der Austrocknungsgeschwindigkeit wurden von jeder der beiden Proben 2 mal 200 g Erde in Glasschalen mit einem Durchmesser von 185 mm gefüllt und nebeneinander im Laboratorium zur Austrocknung bei Zimmertemperatur (ca 20° C) aufgestellt. Nach 24 und 144 Stunden erfolgte die Untersuchung auf den Wassergehalt.

Zwischen Behandlungstermin und dem Tag der Probenahme fielen insgesamt 40,8 mm Niederschlag an 13 Tagen. Am 4. Tage vor der Entnahme der Bodenproben wurden 2,4 mm Regen gemessen. Der letzte stärkere Niederschlag war 15 Tage früher mit 10,5 mm gefallen. Trotzdem der Entnahme der Bodenproben also eine längere nieder-

1) Weiterhin erfolgte eine Bestimmung des Ammonium-Stickstoffgehalts der Bodenextrakte.

Tabelle VIII

Einfluss der DD-Behandlung auf Wassergehalt und Austrocknungsgeschwindigkeit des Bodens. Mittelwerte aus je 2 Parallel-Bestimmungen

	Unbehandelt		Behandelt mit DD	
	%	rel.	%	rel.
Wassergehalt in % Trockenerde, vor Austrocknung	18,70	100	26,50	100
Wassergehalt in % Trockenerde, nach 24 Std. Austrocknung	4,82	25,78	10.45	39,42
Wassergehalt in % Trockenerde, nach 144 Std. Austrocknung	1,46	7,81	2,23	8,41

schlagsfreie Periode vorausgegangen war, und die Proben über 4 Wochen im Laboratorium gelagert hatten, enthielt die mit DD behandelte Erde rund 8 % mehr Wasser, berechnet auf Trockenerde, als der unbehandelte Boden. Durch den primitiven Modellversuch konnte weiterhin nachgewiesen werden, dass auch die Austrocknung der mit DD behandelten Erde erheblich langsamer verläuft. Während nach 24 Stunden die unbehandelte Erde nur noch rund 25 % ihrer ursprünglichen Wassermenge enthielt, betrug dieser Anteil bei der DD-Probe noch 40 %, obgleich infolge des höheren Anfangs-Wassergehalts das Verdunstungspotential im letzten Falle ein bedeutend grösseres war. Auch nach 144 Stunden Austrocknungszeit, als die Erde praktisch den Grad der Lufttrockenheit erreicht hatte, war der Unterschied der beiden Proben im Wassergehalt noch nachweisbar und auch schon rein äusserlich an der Bodenfärbung erkennbar. Da die Austrockungsbedingungen in diesem Versuch sehr extrem waren, — Zimmertemperatur von 19° C und Ausbreitung der Erde in lockerer und dünner Schicht — ist anzunehmen, dass die Wasserabgabe des gewachsenen mit DD behandelten Bodens in noch erheblich höherem Grade verzögert ist, besonders dann, wenn es sich nicht, wie im vorliegenden Falle, um humosen Sand, sondern um schwerere Bodenarten handelt. Wie aus dieser Bestimmung hervorgeht, hat das Mittel offensichtlich die Bodenstruktur im Sinne einer Verschlämmung erheblich verändert.

Auch in der Leitfähigkeit der Bodenextrakte für den elektrischen Strom zeigten sich erhebliche Unterschiede zwischen den beiden Bodenproben. Für den wässrigen Bodenextrakt der behandelten Proben wurde im Leitfähigkeitsmessgerät nach Tödt-Gollnow ein Widerstand von 2135 Ω, für den unbehandelten Boden ein Wert von 4000 Ω gefunden. Aus der mit KCl-Lösungen aufgestellten Eichkurve des Ge-

rätes errechnen sich danach für 100 g Trockenerde für die unbehandelte Probe 14,23 mg, für die behandelte 27,82 mg KCl-Äquivalent. Der so stark erhöhte Elektrolyt-Gehalt des Bodens gibt eine ausreichende Erklärung für die oben angegebene Abweichung dieser Erdprobe im Wassergehalt und im Austrocknungsverhalten.

In der Bodenreaktion, die elektrometrisch mittels Kalomel-Elektrode und Chinhydron-Zusatz gemessen wurde, zeigten sich zwischen behandeltem und unbehandeltem Boden keine Unterschiede; der p_h-Wert der wässrigen Extrakte betrug in beiden Fällen 6,5.

Zur Stickstoffbestimmung wurden jeweils 100 ml des Bodenextraktes mit Schwefelsäure aufgeschlossen und in der Mikro-Kjeldahl-Apparatur unter Zugabe von Natronlauge destilliert; die Titration erfolgte mit n/100 Natronlauge.

Dabei ergaben sich für den unbehandelten Boden 1.62 mg, für den behandelten jedoch 2.17 mg N je 100 g Trockenerde, entsprechend 1.94 mg bzw. 2.60 mg N je 100 ml lufttrockene Erde. Der Extrakt des mit DD behandelten Bodens enthielt somit etwa 34 % mehr Ammonium-N als die Vergleichslösung.

Dieser Befund bestätigt grundsätzlich die Vermutung GRAINGERS (1954), dass DD imstande ist, organisch gebundenen Stickstoff freizumachen und somit den Stickstoffgehalt des Bodens zu steigern. Bei Umrechnung der gefundenen Werte auf die Flächeneinheit — es wurde dabei eine Bodenschicht von 10 cm Tiefe zugrunde gelegt — beträgt der Mehrgehalt des behandelten Bodens 6.58 kg Rein-Stickstoff je ha, entsprechend einer Gabe von etwa 33 kg eines handelsüblichen Mineral-Düngemittels mit 20 % N-Gehalt. Der gefundene Mehrgehalt ist demnach der absoluten Menge nach gering. Das könnte daran liegen, dass die diesbezügliche Wirkung des Mittels nicht voll erfasst worden ist, da der Nitrat-Stickstoff nicht bestimmt wurde und ein Teil des freigesetzten Stickstoffs bereits der Nitrifikation anheimgefallen sein könnte; wahrscheinlicher ist jedoch, dass die quantitativ schwache Wirkung mit dem geringen Gehalt der Versuchserde an organischer Substanz in ursächlichem Zusammenhang steht.

DISKUSSION

Aus den mitgeteilten Beobachtungen ergibt sich, dass sich die Rübenpflanzen der mit DD behandelten Parzellen in dem untersuchten Falle von den Kontrollpflanzen hauptsächlich durch eine ungleiche Welkeanfälligkeit unterschieden.

Dieses für die Gattung *Beta* charakteristische, schon bei relativ ge-

ringen Wasserdefizit-Werten einsetzende Schlaffen der Blätter kann wegen der dadurch ausgelösten weitgehenden Transpirationseinschränkung als xerophytische Anpassung gewertet werden. Die Erscheinung ist jedoch wegen des gleichzeitigen Spaltenschlusses mit einer erheblichen, dem Welkegrad und der Welkedauer proportionalen Verminderung, der photosynthetischen Leistung verbunden (WALTER, 1947). Einer gesteigerten Welkeresistenz, durch die solche Welkeperioden verhindert oder abgekürzt werden, kann somit unter Bedingungen einer erschwerten Wasserbilanz eine erhebliche Bedeutung als wachstumsförderndes Prinzip zukommen. Derartige Verhältnisse waren auf dem Sandboden des Versuchsfeldes und in den ersten niederschlagsarmen Monaten der Vegetationsperiode 1957 bei zeitweilig sehr hohen Tagestemperaturen häufig gegeben. Aus diesem Grunde können die an den nachgebauten Futterrüben des DD-Versuchs festgestellten Unterschiede im Wasserhaushaltsverhalten als hinreichende Ursache der beobachteten Wuchsunterschiede gelten.

Hinsichtlich der kausalen Zusammenhänge zwischen verminderter Welkeanfälligkeit und DD-Behandlung der fraglichen Parzellen scheint es nun sehr wesentlich zu sein, dass alle strukturellen und funktionellen Eigenschaften, durch die sich die Pflanzen der DD-Parzellen von den Vergleichspflanzen unterschieden, den Veränderungen entsprechen, die das Cl-Ion bei einer grossen Zahl von Pflanzenarten, besonders bei der Gattung *Beta*, verursacht (ARNOLD, 1954). Weitere Hinweise dafür, dass die in diesem Falle festgestellte Nebenwirkung des DD auf der Cl-Ionen-Funktion beruhen könnte und der Cl-Gehalt des Mittels, der nach freundlicher Mitteilung von Herrn Dr. HOMEYER, Köln etwa 62,52 % beträgt, in dieser Form wirksam werden kann, sind der stark erhöhte Aschengehalt, der in den Blattspreiten der DD-Pflanzen gefunden wurde und die Steigerung des Elektrolytgehaltes sowie die Verminderung der Wasserverdunstung von Bodenproben, die mit DD behandelt worden waren. Auch diese Wirkungen auf den Aschengehalt der Pflanzen und die Bodeneigenschaften entsprechen spezifischen Eigenschaften des Cl-Ions. Die quantitativen Verhältnisse legen ferner die Annahme einer Cl-Wirkung des DD auf Boden und Pflanze nahe. Bei einer Anwendung von 89 ml/qm und einem spez. Gewicht von 1,2 (MC BETH, 1957) = 96 g/m² beträgt die damit in den Boden gebrachte Cl-Menge 600 kg je ha. Dies entspricht einer Gabe von etwa 10 dz Natriumchlorid bzw. 12 dz 40 % Kalidüngesalz = 480 kg K_2O je ha, also einer extrem hohen Kalidüngung (als übliche Norm können 200 kg K_2O je ha bei Futter- und

Zuckerrüben gelten). Dabei ist zu bedenken, dass in der Regel nach der DD-Anwendung auch noch die übliche Kalidüngung in der Chloridform verabreicht wird. Bei diesen Mengenverhältnissen ist verständlich, dass noch im zweiten Vegetationsjahre nach der Behandlung eine auf das Pflanzenwachstum sich auswirkende Menge von Cl-Ionen im Boden zurückgeblieben sein könnte.

Die Wirkung der DD-Behandlung auf die Bodenfeuchtigkeit, besonders auf die Verdunstungskraft des Bodens, die in den Untersuchungen an behandeltem Boden festgestellt wurde, kann das Pflanzenwachstum besonders im ersten Vegetationsjahr nach der Behandlung auch unmittelbar beeinflussen, nämlich positiv auf leichten wasserarmen Böden durch längere Erhaltung des Winterfeuchtigkeitsvorrates, negativ auf schweren, kolloidalen und zu stauender Nässe neigenden Böden durch zusätzliche Erschwerung der Austrocknung. Eine bessere Wasserversorgung als direkte Ursache der Wachstumsförderung ist in dem vorliegenden Fall jedoch nicht anzunehmen, da die Futterrüben in zweiter Tracht nach der Behandlung standen und Blattstruktur und Welkeverhalten der Pflanzen durch erhöhten Wassergehalt des Bodens im entgegengesetzten Sinne verändert werden, wie es bei den Pflanzen der DD-Parzellen der Fall war.

Aus den gleichen Gründen ist auch eine bessere N-Ernährung als unmittelbare Ursache der Wachstumssteigerung unwahrscheinlich, da eine bessere Versorgung mit diesem Nährstoff ebenfalls Oberflächenentwicklung und Welkeanfälligkeit der Pflanzen steigert. Auch waren die für eine ungleich starke N-Versorgung typischen Unterschiede im Gehalt der Pflanzen an löslichem Stickstoff bei den Futterrüben der behandelten und unbehandelten Parzellen nicht festzustellen. Dafür, dass eine solche Beziehung, die in anderen Fällen auch vermutet wurde (GRAINGER, 1954), grundsätzlich möglich ist, gaben die Stickstoff-Untersuchungen an Extrakten DD-behandelter Erden gewisse Hinweise.

Die Wirkung des DD auf das Wasserhaushaltsverhalten der Pflanzen stellt eine der möglichen Formen einer Nebenwirkung des Mittels dar, die als wachstumsfördernder Effekt an bestimmte Voraussetzungen geknüpft ist, nämlich an einen bestimmten Konzentrationsbereich des wirksamen Mittelbestandteils im Boden, d.i. mit grosser Wahrscheinlichkeit an eine ausreichende Cl-Ionen-Menge in der Bodenlösung, ferner an eine entsprechende Reaktion der Pflanzenart, wobei sich chlorindifferente und chlorempfindliche Pflanzen unter gleichen Umständen anders verhalten als chlortolerante und schliesslich an

Standorts- und Witterungsbedingungen, unter denen sich eine verminderte Welkeanfälligkeit überhaupt wachstumsfördernd auswirken kann. Auf sehr gut mit Wasser versorgten Böden oder bei einer hohen und gleichmässig verteilten Niederschlagsmenge, wo Welkeperioden nicht auftreten, macht sich naturgemäss eine verminderte Welkeanfälligkeit auf das Wachstum nicht geltend. Es kann daher nicht erwartet werden, dass in allen Fällen der DD-Anwendung die gleiche Reaktion, die an den Futterrüben festgestellt werden konnte, eintritt. Unter andersartigen Bedingungen könnte der Chlorgehalt des Mittels auch ganz andere Folgen haben, z.B. eine Zunahme der Bodenazidität durch Kalkverluste infolge der Cl-Anreicherung im Boden und demzufolge eine Wachstumsdepression säureempfindlicher Pflanzen (Klee, Luzerne u.a.), während der gleiche Effekt auf alkalischen Böden mit Mangel an Spurenelementen deren Löslichkeit steigern und sich wiederum pflanzenertragssteigernd auswirken könnte. So wenig demnach die mitgeteilten Befunde an den Futterrüben verallgemeinert werden dürfen, so sehr zeigt dieses Beispiel, dass mannigfaltige Nebenwirkungen des Nematizids auf Boden und Pflanze möglich sind.

SUMMARY

The secondary action of Shell D-D and similar compounds based on Dichloropropen, over and above their nematicidal properties, has frequently been referred to in the literature, and takes the form of growth stimulation in crops cultivated after soil treatment with these compounds. Stimulation was investigated in an instance in which increased growth occurred in fodder beet in 1957. The field was treated with DD in 1955 to control potato-root eelworm, potatoes were grown in 1956, and fodder beet in 1957. Species of nematodes injurious to sugar beet were not found in the treated or untreated plots. Leaves from DD treated plots compared with those from untreated plots possessed a higher water content, lower osmotic pressure in the sap, a lower water deficit, greater succulence, a smaller surface area, and a raised ash content.

In wilting experiments the fall in water content, and the rise in osmotic pressure and water deficit were slower than in plants from untreated soil. Recovery from wilting was quicker and more marked. Under the local weather and other conditions, the difference in wilting properties of the plants from treated and untreated soil would account for the growth differences.

Three months after injection, the soil treated with DD in October 1957 showed a higher electrolyte, water and ammonium nitrogen content, and reduced evaporating power. Since the qualitative changes in plants and soil brought about by DD correspond to the specific functions of Cl-ions, it is very probable that the secondary action of this nematicide is due to its chlorine content.

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Eingegangen: 4. Februar, 1958

Nematologica III (1958): 229-235. E. J. Brill, Leiden

APHELENCHOIDES DACTYLOCERCUS N.SP. AND *A. SACCHARI* N.SP. (NEMATODA: APHELENCHOIDEA)

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During recent research into nematode parasites of mushrooms, the following two new species of *Aphelenchoides* were encountered which were capable of destroying the mushroom mycelium. Both of these species closely resemble *A. parietinus* as redescribed by FRANKLIN (1955).

Measurements given in the following descriptions were made on specimens relaxed by gentle heat in water, fixed in TAF (COURTNEY, POLLEY & MILLER, 1955), stained in cotton blue lactophenol and mounted in glycerine (GOODEY, 1957). Observations were made on living and preserved specimens. Mounts of transverse sections of the body and heads for end-on view were made in glycerine jelly and also in methyl cellulose (CAIRNS & TARJAN, 1955).

APHELENCHOIDES DACTYLOCERCUS N. SP.

(Fig. 1, A-F)

25 ♀♀: L = 445 μ (s = $\pm$ 20 μ) (measured range 414-502 μ) a = 26.1 (22.2-31.0); b = 8.1 (7.3-9.1); c = 10.5 (8.6-11.8); V = ^{30} 67 % $^{4.1}$ ($^{23-39}$ 66-70 $^{1.9-5.0}$). Holotype: L = 444 μ; a = 26.8; b = 7.8; c = 10.3; V = ^{34} 68 % $^{3.2}$.

Body cylindrical, tapering at both ends. Cuticle finely anulated, the annules 0.8-1.0 μ wide in mid body region. Lateral fields prominent, marked by three incisures, occupying between a quarter and a fifth of the total body-width (Fig. 1 E & F). Lateral fields begin about halfway between head and oesophageal bulb and end midway between anus and tail terminus. Head slightly offset, without annulations. An end-on view shows six lips but no papillae or amphidial openings are visible (Fig. 1 C). Stylet 10 μ long (n = 10) made up of the usual two parts with distinct basal thickenings (Fig. 1 B & D). Stylet tip surrounded by guide ring which appears star-shaped when seen from the front

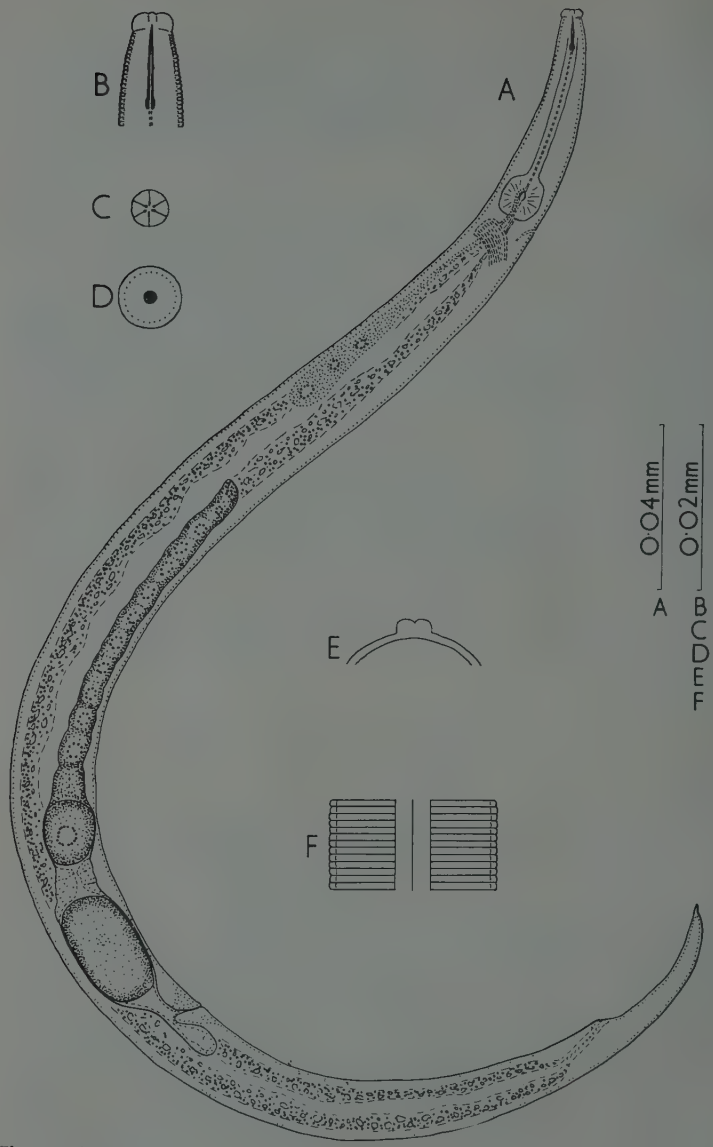

Fig. 1. *Aphelenchoides dactylocercus*. A. Female nematode; B. lateral view of head; C. End-on view of head; D. Section across head at stylet base; E. Section in mid-body region showing lateral field; F. Lateral view of cuticle in mid-body region.

and as two short rods in lateral view. First region of oesophagus narrow with well marked lumen followed by prominent, muscular, median bulb in the centre of which is a conspicuous valve with crescentic thickenings. Elongated oesophageal glands lying dorsally along intestine and about three body-widths long: dorsal gland duct opening into lumen of oesophagus just in front of valve. Intestine begins as tube immediately behind median bulb, widens gradually and runs straight to narrow rectum which is twice as long as the anal body-width. Anus distinct; behind it the tail narrows suddenly and then gradually tapers to the tip: tail over four times as long as the anal body-width. Tail terminus convex-conoid with sharply pointed, ventrally placed, mucro. Tail curves ventrally when relaxed by gentle heat and has a finger like appearance; hence the specific epithet of *dactylocercus*. Nerve ring situated just behind median oesophageal bulb. Excretory pore on ventral surface usually just in front of nerve ring but may be as far forward as centre of median bulb. Ovary outstretched, consisting of a single row of about twelve oocytes. Oviduct short, only one egg seen at a time in the uterus. Eggs just over two and a half times as long as broad (12.5 × 30.5 μ). Post vulval sac short, usually less than one body-width long. Vagina directed obliquely backwards. Vulva transverse slit about one third of a body-width long.

No males have been found.

Type habitat: Pot of soil growing young Sitka spruce trees, *Picea sitchensis* (Borg.) Carr. — Nematology Department, Rothamsted.

Type slide: Holotype ♀ slide no 86/17/1 in the collection of the Nematology Department, Rothamsted Experimental Station.

APHELENCHOIDES SACCHARI N. SP.

(Fig. 2, A-E)

25 ♀♀: L = 620 μ (s = ± 68 μ) (measured range 510-750 μ) a = 25.8 (19.8-33.8; b = 11.4 (9.7-14.3); c = 18.0 (13.8-24.7); V = ^{48} 70 % ^{12} ($^{36\text{-}56}$ 67-72 $^{10\text{-}15}$).

25 ♂♂: L = 540 (s = ± 54 μ) (measured range 450-650 μ) a = 24.8 (20.1-27.7); b = 9.8 (8.6-11.4); c = 16.6 (13.0-24.8); T = 75 % (66-88).

Holotype ♀: L = 615 μ; a = 26.8; b = 10.5; c = 18.1; V = ^{48} 69 % ^{13}.

Allotype ♂: L = 560 μ; a = 22.6; b = 10.0; c = 15.2; T = 73 %.

Body cylindrical, tapering at both ends. Cuticle annulated, annules

0.85-1.0 μ wide. Lateral fields prominent and narrow, marked by three incisures, occupying between one fifth and one seventh of the total body-width. Lateral fields begin midway between head and median bulb and end just before tail terminus. Head offset, without annulations. An end-on view shows six lips but no amphidial openings or papillae visible. Stylet 11.4 μ long (n = 7) made up of the usual two parts with distinct basal thickenings (Fig. 2 B). Stylet tip surrounded by guide ring which appears as two short rods in lateral view and star-shaped when seen from the front. Alimentary tract similar to *A. dactylocercus*, but dorsal oesophageal gland ends in a rather wide lobe occupying about half the body-width at that point. Nerve ring about one half body-width behind median oesophageal bulb. Excretory pore on ventral surface opposite or just in front of the nerve ring.

Female: Tail short and rather wide, about three anal body-widths long. Anus distinct. Tail tapers gradually to bluntly rounded terminus with ventral mucro. Ovary outstretched, sometimes reaching almost to median oesophageal bulb. Oocytes arranged in tandem except for a short distance at anterior end where they appear in a double row. Oviduct two to three body-widths long. Usually only one egg at a time in the uterus, although a few specimens have been seen with three eggs, one egg occupying the post vulval sac. Eggs two and a half times as long as broad (15 × 38 μ). Post vulval sac extends about half the distance between vulva and anus. Vagina directed obliquely backwards. Vulva a transverse slit one third body-width long.

Male: Slightly smaller than female. Tail curves ventrally through about 130° when relaxed by heat; slightly longer than female tail with more conoid terminus bearing a truncate mucro. Three pairs of subventral papillae: one pair adanal, one just anterior to middle of tail and one subterminal. Testis single and outstretched. Spicules paired, slightly built and rather small: dorsal limb measuring 15-17 μ (n = 6). Both limbs of spicules curve ventrally but tend to flatten towards distal end (Fig. 2 C).

Males occur in ratio 1 : 50 females on mushroom plate cultures.

Type habitat: Rotting roots of Sugar Cane (*Saccharum officinale* L.) from Jamaica.

Type slides: Holotype ♀ slide no. 86/18/1; allotype ♂ slide no. 86/18/2 in the collection of the Nematology Department, Rothamsted.

Bionomics: Both *A. dactylocercus* and *A. sacchari* are easily cultured in large numbers on malt agar plates with mushroom mycelium (*Agari-*

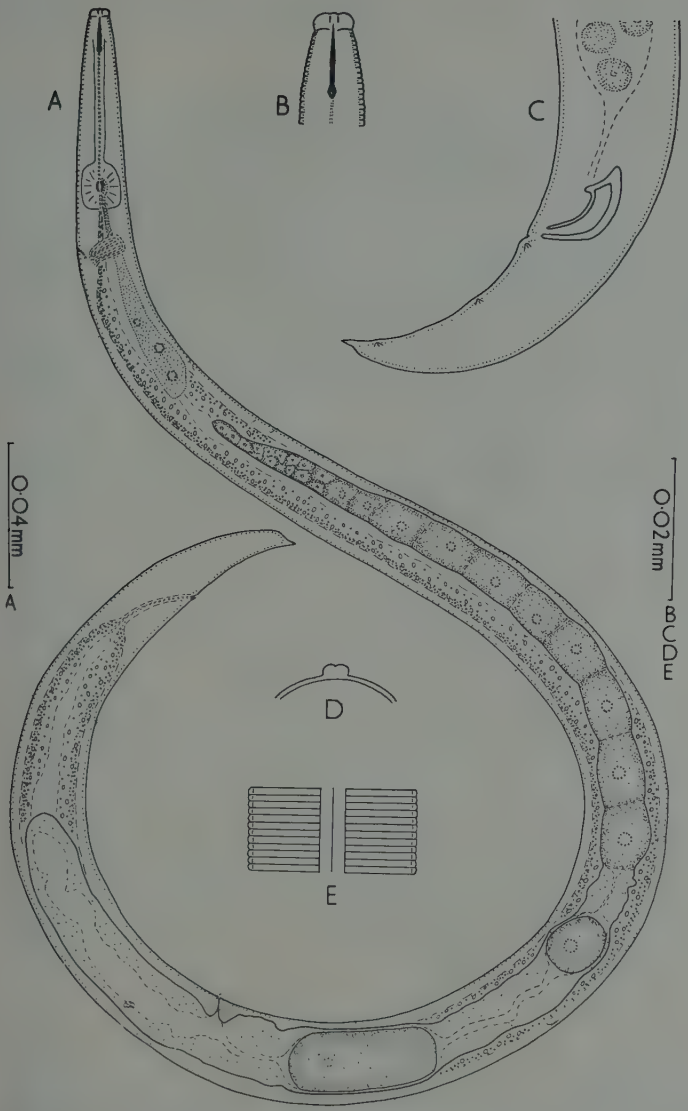

Fig. 2. *Aphelenchoides sacchari*. A. Female nematode; B. Lateral view of head; C. Male tail; D. Section in mid-body region showing lateral field; E. Lateral view of cuticle in mid-body region.

cus hortensis Cooke). They will also live on fungal plates of *Alternaria tenuis* Nees but they do not thrive as well on this medium as they do on mushroom.

RELATIONSHIPS

A. dactylocercus n.sp. is very similar to *A. parietinus* (Bastian, 1865), Steiner, 1935 as redescribed by Franklin, 1955. However it differs from the latter in having a longer and more pointed tail and only three incisures on the lateral field. The eggs also are two and a half times as long as broad (four times as long as broad in *A. parietinus*). *A. dactylocercus* also resembles to some extent *A. composticola* Franklin, 1957, *A. hunti* Steiner, 1935 and *A. minimus* Meyl, 1953. It differs from the first two species in its smaller size, much smaller post-vulval sac and tail shape. It differs from *A. minimus* in its larger size and body proportions, shorter post-vulval sac and wider body annules.

A. sacchari n.sp. differs from *A. parietinus* (sensu stricto) in the number of incisures on the lateral field, larger post-vulval sac, smaller egg size, shorter tail and shorter distance between head and median oesophageal bulb. From *A. saprophilus* Franklin, 1957, *A. composticola* Franklin, 1957 and *A. cyrtus* Paesler, 1957 it differs in having a shorter and differently shaped female tail, smaller and differently shaped spicules, shorter oesophagus and, in the case of *A. saprophilus*, three instead of four incisures on the lateral field.

Franklin (1955), redescribing *A. parietinus*, suggests that some of the nematodes which have been synonymised with it should, in fact, be considered as distinct species. The two new species described above have therefore been compared with the descriptions of all the species synonymised with *A. parietinus* by T. Goodey (1951) and with the species mentioned by Franklin, (1955). Most of these descriptions are meagre but none were found with which either of the above new species could be identified.

The writer wishes to thank Dr. M. T. Franklin and Dr. J. B. Goodey for help and advice.

ZUSAMMENFASSUNG

Aphelenchoides dactylocercus n.sp. und *A. sacchari* n.sp. werden beschrieben und abgebildet. Beide sind fungivor und leicht auf Pilzmyzel (*Agaricus hortensis* Cooke) zu kultivieren.

Die hauptsächlichsten diagnostischen Eigenschaften von *A. dactylocercus* n.sp. sind der schmale fingerförmige Schwanz, der kurze postvulvare Sack und die

drei Incisuren auf dem Seitenfeld, während *A. sacchari* n.sp. drei Incisuren auf dem Seitenfeld, einen kurzen Oesophagus, einen kurzen Schwanz beim Weibchen und eine geringe Grösse der Spicula aufweist.

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Received for publication: January 24, 1958

Nematologica III (1958): 236-243. E. J. Brill, Leiden

OBSERVATIONS ON THE EMERGENCE FROM CYSTS AND THE ORIENTATION OF LARVAE OF THREE SPECIES OF THE GENUS *HETERODERA* IN THE PRESENCE OF HOST PLANT ROOTS

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Much work has been published on the influence of root diffusates on the emergence *in vitro* of larvae from cysts of different species of the genus *Heterodera*. There are also numerous references in the literature to larval emergence in the soil in the presence of host and non-host plants. There is, however, little information about larval emergence in the presence of plants where there is control of the environmental conditions. The question whether larvae of the genus *Heterodera* are attracted to roots has also received little attention, the only work in this connection being on the related genus *Meloidogyne* (WIESER, 1955, 1956). The work herein described is an attempt to show how pressure deficiency (or suction) and moisture content in sand influence the rate of larval emergence from cysts of *Heterodera major* O. Schmidt, *H. schachtii* Schmidt and *H. rostochiensis* Wollenweber. The influence of the host plant on this relationship is examined and the problem of attraction of larvae to roots has also been studied.

MATERIALS AND METHODS

Cysts of *H. schachtii* and *H. rostochiensis* were recovered from infested soil by a method already described (WALLACE, 1957). Cysts of *H. major* were obtained by a technique devised by HESLING (1956).

The apparatus for the emergence and attraction experiments is shown in Fig. 1. It consists of a sintered-glass funnel containing sand which had been sieved to a given particle size range of 250-600 μ. The sand was washed to remove as much clay as possible and poured into the funnels to a depth of 3 cm. By adjusting the height of the water meniscus in the side tube, the suction in the sand was controlled, the moisture characteristic of the sand giving the relationship between suction and moisture content (WALLACE, 1958). A series of such

funnels with sand at different suctions, but at constant temperature, was set up. Cysts were introduced into the sand at a depth of one cm along a diameter of the sintered-glass plate and host plant seeds were sown at a depth of 3 mm in a line parallel to the cysts and 2.5 cm away as shown in Fig. 1. The top of the funnel was covered with a sheet of polythene. The number of seeds and cysts and the number of replicates for each suction varied according to the *Heterodera* species being studied. For *H. major* five oat seeds and twenty cysts with eight-fold

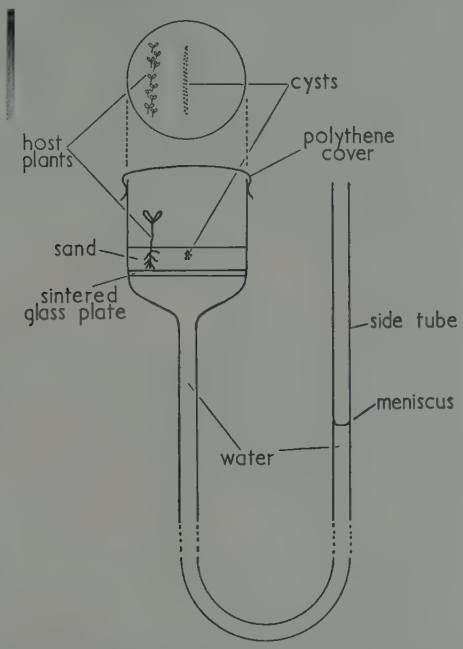

Fig. 1. Diagram of the apparatus used in the study of larval emergence from cysts and their attraction to host plant roots.

replication were used at each suction. For *H. schachtii* thirty cress seeds, 100 cysts and five-fold replication were employed. With *H. rostochiensis* fifteen tomato seeds, 100 cysts and six-fold replication per suction were used. The duration of the experiment was 7 days for *H. major* and *H. schachtii* and 11 days for *H. rostochiensis*. Seeds were allowed to germinate on damp filter paper before they were introduced into the sand. At the end of the experiment a rectangular piece of thin sheet-brass was pushed vertically into the sand along the diame-

ter containing the cysts until it rested on the sintered-glass plate. By this means it was possible to divide the sand in the funnels into two halves, one of which contained the host plants. The sand from each side was scooped out and placed on a sieve of 80 meshes/inch and the larvae washed through, collected in a petri dish and counted. Larvae which remained on the plate after removal of the sand were recovered by turning the funnel so that the plate was vertical and then washing each half in turn. By this means it was possible to determine the total number of larvae which had emerged from the cysts and the number of larvae on each side of the diameter containing the cysts. The two sets of data gave an estimate of the rate of larval emergence of the cysts and the percentage number of larvae which occurred in the half of the funnel containing the plants, which was taken as a measure of the attraction of the larvae to the plants. After each experiment the funnels were cleaned by passing alcohol, then hydrochloric acid and finally water through the sintered glass plates under suction; the water in the apparatus was renewed at the same time.

RESULTS

The results of the experiments are given in Fig. 2. The curves for larval emergence and for larval attraction are given for each of the three species. In order to simplify the graphs the moisture characteristic of the sand has been included in the results for *H. major* only and the values of moisture content have been omitted. The range of moisture content was 5-30 % by volume. The emergence curves in the presence and in the absence of host plants are included in the same graph to facilitate comparison. In the attraction curves, the 50 % line has been drawn to indicate the point where neither attraction nor repulsion is present. The following conclusions were drawn from the data. Stimulation of larval emergence from cysts in the presence of host plant roots occurs in *H. schachtii* and *H. rostochiensis*; there is a suggestion of stimulation of *H. major* at 5 and 10 cm of water suction. In this paper stimulation is said to occur when the rate of larval emergence is above that in the absence of roots. Thus, in the case of *H. major* there is strong evidence that at suctions greater than 10 cm water inhibition of larval emergence occurs. The larval emergence curves in the absence of roots for *H. major* and *H. schachtii* both show an optimum suction for emergence but the decline in the emergence rate at higher suctions is less for *H. major* than for *H. schachtii*. The emergence rate of *H. rostochiensis* in the absence of roots was so low that

no influence of suction was detected. The form of the emergence curve in the presence of roots is similar for all three species and appears to be related to the moisture content of the sand since the curves resemble the moisture characteristic in shape. Attraction of

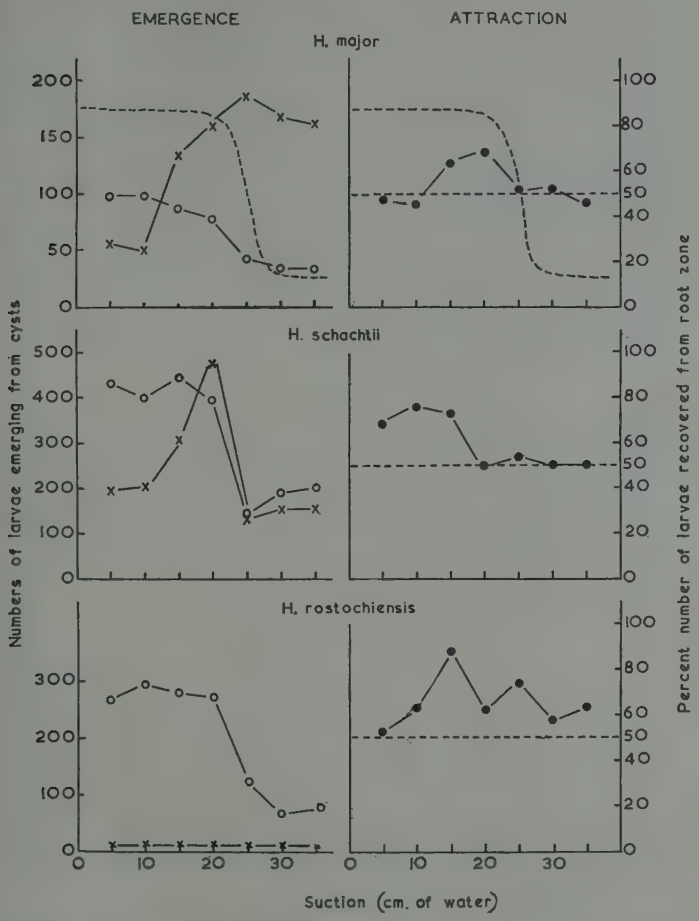

Fig. 2. Larval emergence and attraction in sand for three species of *Heterodera*. The moisture characteristic of the sand is indicated by a dotted line in the graphs for *H. major*. Crosses indicate larval emergence in the absence of plants, open circles in the presence of host plants. Closed circles indicate the percentage of larvae attracted to host plants. Each point is the mean of a number of replicates. There were eight replicates for *H. major*, five for *H. schachtii* and six for *H. rostochiensis*.

larvae to roots of the host plant occurs in all three species. There appears to be an optimum suction for attraction between 10-20 cm of water.

An experiment was also performed in which a constant suction of 15 cm of water was maintained in the sand but the distance between the cysts and the plants was varied. Only *H. schachtii* cysts were used in this experiment and the methods were the same as those described previously. The results are shown in Fig. 3. As the distance between cysts and cress plants increased the rate of larval emergence decreased until at a distance of 4.5 cm it approached the emergence rate in the absence of plants. Attraction to roots occurred at all suctions.

In a further experiment cress seed was grown on one side of the funnels for one week and then the sand and cress washed out of the funnels with water. Clean sand was then placed in the funnel, cysts of *H. schachtii* introduced and the suction adjusted to 15 cm of water. Ten funnels were set up in this way and at the end of a further week

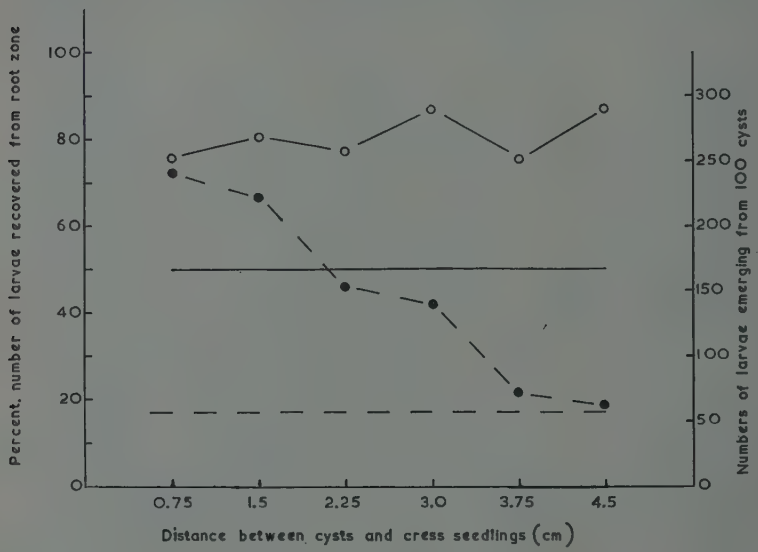

Fig. 3. Larval emergence and attraction in sand for *H. schachtii* at a constant suction of 15 cm of water but with different distances between cysts and host plant. Continuous lines and open circles indicate percentage attraction, discontinous lines and closed circles indicate larval emergence. The horizontal continuous line is the 50% line indicating no attraction, the horizontal dotted line gives the larval emergence in the absence of plants at 15 cm of water suction. Each point is the mean of 5 replicates.

the percentage number of larvae in the half of the funnel where cress plants had previously grown was determined. The results are given in Table I. The data suggest that larval attraction occurs in the absence

TABLE I

The attraction of larvae of Heterodera schachtii in the absence of plant roots

Funnel	Numbers in non-root half of funnel	Numbers in root half of funnel	Total	% of total in root half of funnel
1	59	105	164	64.0
2	49	129	178	72.5
3	64	358	422	84.8
4	38	212	250	84.8
5	36	204	240	85.0
6	68	278	346	80.3
7	72	502	574	87.5
8	40	272	312	87.2
9	29	157	186	84.4
10	77	187	264	70.8

of roots. The fact that evidence of attraction disappeared if the plates were cleaned with alcohol and acid before the fresh sand and the cysts were introduced, suggests that some chemical secreted by the roots remains in the sintered glass plate after the cress had been removed. It is possible that this chemical then influenced the movements of larvae when they were subsequently introduced into clean sand.

DISCUSSION

The form of the larval emergence curves in the presence of plants is probably the result of numerous inter-related factors, and so the data do not show how each of these factors influences the emergence rate. It is suggested that four of the factors which produce the net result given in the graphs are (1) the concentration of root diffusate secreted by the roots. The concentration factor will be dependent on the moisture content of the sand; at the higher suctions the diffusate will be more concentrated than at lower suctions. The influence of diffusate concentration on larval emergence has been described for *H. rostochiensis* (FENWICK, 1952) and for *H. schachtii* (WALLACE, 1956). (2) The rate of diffusion of the root-diffusate from roots to cysts. The experiment on the influence of distance of the host plants from the cysts on emergence rate suggests that a diffusion factor is present. It is suggested that the rate of diffusion of the diffusate will

decrease as suction increases and moisture content decreases. (3) Gradients of moisture content in the sand caused by uptake of water by the roots. Gradients of moisture content created by rapid uptake of water by the roots would influence both concentration and diffusion of the root diffusate and might create suctions in the sand which would inhibit emergence. It is possible that the inhibition of emergence of *H. major* shown in Fig. 2 is a result of this effect. (4) The inhibition of larval emergence at high suctions caused by thin water films at the oral and vulval openings of the cyst. This phenomenon has been described for *H. schachtii* (WALLACE, 1955).

The shape of the emergence curves is probably the result of these and other factors working together and illustrates how the interpretation of results is difficult when plants are used in an experiment. The salient point which arises from the experiments is that moisture content of the sand influences to a great extent the larval emergence rate in the presence of plants.

The fact that larvae occur mostly on the root side of the funnels after a given time gives no indication of the mechanism of attraction. Observations on larvae in the vicinity of roots in single sand layers, showed that they tend to remain in the close vicinity of the root when they have once contacted it. Thus, if the larvae were moving at random among the sand particles they would eventually contact a root and then tend to stay there. In time a majority of the larvae would accumulate in the root zone. Such a means of attraction precludes the idea that the roots can exert an influence on the larvae at a distance. However, the results given in Table I show that larvae of *H. schachtii* are attracted to one side of the funnel in the absence of plants. It is, therefore, suggested that larvae can orientate themselves to an attractant some distance away and that the mechanism of attraction is by orientation to a concentration gradient of some chemical substance secreted by the plant roots. The form of the curves in Fig. 2 shows that there is an optimum range of suction for attraction. This may be due to the influence of moisture content on larval movement. At high and low suctions movement is inhibited but maximum mobility is attained in the intermediate suctions (WALLACE, 1958). Thus, in experiments involving the attraction of larvae to plants it is possible that larvae may only respond to a concentration gradient if their mobility in the medium is sufficiently high.

ZUSAMMENFASSUNG

Bei Anwesenheit von Wirtspflanzenwurzeln tritt ein Schlüpfen der Larven aus Cysten von *Heterodera major, H. schachtii* und *H. rostochiensis* ein. Die larvale Schlüpfrate in Sand wird in grossem Umfange vom Feuchtigkeitsgehalt beeinflusst, die einzelnen wirksamen Faktoren wurden jedoch nicht geklärt. Bei niedrigem Feuchtigkeitsgehalt wurde das Larvenschlüpfen aus den Cysten von *H. major* gehemmt. Die Anlockung der Larven durch die Wurzeln der Wirtspflanzen tritt bei allen drei *Heterodera*-Arten auf. Die Ergebnisse lassen erkennen, dass sich die Larven zu einem Konzentrationsgradienten einer chemischen Substanz orientieren, die von den Pflanzenwurzeln ausgeschieden wird. Die Reaktion auf einen solchen Gradienten kommt mehr zum Ausdruck, wenn die Larven eine hohe Beweglichkeit im Sand haben.

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Received for publication: February 6, 1958

Nematologica III (1958): 244-256. E. J. Brill, Leiden

STUDIES IN THE RELATIONSHIP OF EELWORMS AND BACTERIA TO CERTAIN PLANT DISEASES.
II. FURTHER ANALYSIS OF THE STRAWBERRY CAULIFLOWER DISEASE COMPLEX

BY

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Several different symptoms are associated with the infestation of field strawberries in Great Britain by the leaf and bud nematodes (*Aphelenchoides ritzema-bosi* (Schwartz) and *A. fragariae* (Ritzema Bos)), including feeding areas, enations, alaminate leaves, red plant and cauliflower. The present paper is concerned mainly with the etiology of cauliflower and alaminate leaves.

"Alaminate leaves" are inconspicuous, usually occurring as a rosette of very small, mainly glabrous petioles about ½-1 cm in length, often browned superficially and tapered to a point which represents an extremely reduced lamina. Their appearance in a primary crown is usually followed by the proliferation of secondary crowns, some of which may develop similar structures. Alaminate leaves are relatively impermanent and they, and the primary crown bearing them, usually die, forming "open-centre" plants. Occasionally however, the crown does not die, but develops cauliflower symptoms.

"Cauliflower" is symptomatic of a more fundamental disturbance of the growth-regulatory system of the plant, resulting in the continued proliferation of axillary buds on affected crowns. In extreme forms such crowns may be reduced to stunted fleshy rosettes consisting mainly of leaf tissue in the form of short swollen petioles. Between this and the healthy plant are intermediate forms for which the term 'near-cauliflower' has been used.

Alaminate leaves were first described from experimental plants inoculated with *A. ritzema-bosi* but, as a stage in the development of the open-centre plant, they were subsequently found to be the most widespread and common manifestation of *Aphelenchoides* infestation of field strawberries. Cauliflower, on the other hand, is not very common and even in the most susceptible varieties, such as Mme. Lefebvre, it rarely occurs in more than 1 % of the eelworm-infested plants.

Cauliflower disease was originally attributed by RITZEMA BOS (1889) to the eelworm *Aphelenchoides* (syn. *Aphelenchus*) *fragariae*, but subsequent attempts to reproduce it by inoculation were consistently unsuccessful (LEES & STANILAND, 1925; STANILAND, 1926; STANILAND & SWARBRICK, 1929; DICKER, 1948). At a later stage LACEY (1939) implicated *Corynebacterium fascians* (Tilford) Dowson in cauliflower disease, but inoculations with this organism, already shown to be the sole cause of leafy galls in other plants, were inconclusive. The invariable association of both *C. fascians* and *Aphelenchoides* spp. with cauliflowers was confirmed by us and lead us to believe that these two agents might be jointly concerned in the disease. This hypothesis was confirmed experimentally. Cauliflowers were induced in potted strawberry runners by inoculating eelworms and bacteria together but not with either separately (CROSSE & PITCHER, 1952). The strain of the bacterium was found to be important, only those originating from strawberry cauliflowers being effective. *C. fascians* alone produced no response but alaminate leaves developed wherever the eelworm was inoculated, irrespective of whether the bacterium was co-inoculated or not. This suggested that the eelworm might be the sole cause of alaminate leaves, but this conclusion was later placed in doubt by the discovery of 'wild' strains of *C. fascians*, or an organism not distinguished from non-pathogenic strains of *C. fascians*, in all the experimental plants including the uninoculated controls. These wild strains were found to be generally less virulent than those isolated from strawberry cauliflower, and on this basis the following provisional conclusions were reached on the etiology of the disease complex:

1. Cauliflower was mainly due to the activity of highly virulent strains of *C. fascians* with the eelworm playing a subsidiary but essential role, probably as vector carrying the bacterium into the meristematic regions of the crown normally inaccessible to it because of their tight enclosure in stipules.
2. Alaminate leaves were due either to the eelworm alone, or to the eelworm in association with strains of *C. fascians* of low virulence to strawberry.

The main object of the present investigation has been (1) to define more precisely the relative roles of the eelworm and bacterium in the etiology of cauliflower and (2) to determine to what extent, if any, the bacterium was concerned in the formation of alaminate leaves. It

was obvious that the key to these problems lay in determining the effect of the eelworm on strawberry plants in the absence of *C. fascians*. The experiments described below have been directed towards this end.

EXPERIMENTAL PROCEDURE

Further experiments with potted runner plants of the variety Mme Lefebvre confirmed the joint role of eelworms and *C. fascians* in the etiology of cauliflower disease, but attempts to exclude contaminant wild strains of the bacterium by striking plants into autoclaved soil through a layer of autoclaved sand, and by watering the plants with bactericides before and after inoculation, were unsuccessful. It was finally concluded that further progress in the analysis of the disease complex required methods permitting the axenic culture of eelworms on strawberry plants. This raised two problems, namely 1) the establishment and maintenance of actively growing plants under aseptic conditions, and 2) the preparation of bacteria-free eelworm inoculum.

Growth of plants under aseptic conditions. The wild strawberry *Fragaria vesca*, which can be readily raised from seed, was found to respond to inoculations with eelworms and bacteria with the same range of symptoms as the cultivated strawberry. It was therefore chosen for the investigations described below. Seeds were sterilised in a solution containing 0.1 % mercuric chloride and 0.01 % of the wetter dioctyl sodium sulphosuccinate, or alternatively they were placed in the wetter, washed, and then sterilised in a freshly made 2 % solution of chloramine-T. Seedlings were raised in autoclaved glass vessels containing either soil or an inorganic nutrient agar medium. Under conditions of good illumination in the greenhouse or laboratory, seedlings usually reached a size suitable for inoculation in 2-3 months.

Preparation of bacteria-free eelworms. A. ritzema-bosi was used exclusively in these investigations and, as in previous experiments, was sterilised by immersion for 72 or 96 hours in a solution of 3.75 p.p.m. ethoxy ethyl mercury chloride and 0.01 % dioctyl sodium sulphosuccinate (Crosse & Pitcher, 1952, 1953). This method has been further developed and tested and has frequently given complete sterility in batches of approximately 200 eelworms. No improvement was obtained by using 0.1 % streptomycin sulphate (potency 720 i.u./ml) either as an alternative or additional serial treatment and there was evidence that streptomycin alone was not effective against fungal contaminants. Ethoxy ethyl mercury chloride solution has been suc-

cessfully used by Lownsbery & Lownsbery (1956), who completely sterilised 4 out of 12 batches of 1000 larvae of *Meloidogyne incognita* var. *acrita*. Their suspensions contained 1 % of saprophagous nematodes (*Rhabditida*) which may have added to the difficulty of obtaining sterility. Fenwick (1956) however, found ethoxy ethyl mercury chloride at 5 to 20 p.p.m. killed larvae of *Heterodera rostochiensis* in 24 hours, so this substance may not be universally applicable as an eelworm sterilant.

Details of four experiments based on the above methods are described below.

SEEDLINGS GROWN IN STERILISED SOILS

Experiment 1. In this the effect on *F. vesca* seedlings of the following inoculation treatments was tested:

(a) eelworms
(b) *C. fascians*
(c) eelworms + *C. fascians.*
(d) none

Seedlings were raised in glass preserving jars approximately 15 cm in height and 11 cm in diameter. A short piece of wide glass tube was sealed into a circular hole in the lid with a sleeve of thick-walled rubber tubing (Fig. 1). The bottom of the jar was covered to a depth of 3 cm with a mixture of loam, sand and activated charcoal, in the proportions by volume of 20:10:3 respectively. The addition of charcoal considerably improved subsequent growth of the seedlings. The lids of the jars were sealed with spring clips, the glass tube insert plugged with cotton wool, and the whole container then autoclaved at 15 lbs/30 minutes. When cool, 50 ml of sterile distilled water was added to each bottle from an automatic pipette with a hooded jet. Seeds were sterilised in mercuric chloride for 10 minutes at atmospheric pressure and washed several times in sterile water. The seeds were spread out in a sterile dish and transferred to the jars when the radicle began to show. The jars were placed in a greenhouse in early September and the seedlings inoculated about 2½ months later; by this time they were in approximately the fourth leaf stage and varied in height from 2-5 cm.

Eelworms were extracted from chrysanthemum leaves and sterilised in bulk in a petri dish for 72 hours. The suspension was then transferred to corked, sterile, 3 ml centrifuge tubes, washed four times in sterile water, allowed to stand overnight, and finally washed twice more. The plants were brought into a culture room and each inoculated

with approximately 25 eelworms in about 0.01 ml of water. The inoculum was taken up in the tapered end of a sterile glass tube, and then gently expelled into the axil of a leaf by blowing. Inoculations with *C. fascians* were made on the following day with a drop of suspension delivered from a sterile pipette into the crown of the plant. The strain of the bacterium used was C6R, originally isolated from a cauliflower crown of a cultivated strawberry, variety Mme Lefebvre; the inoculum consisted of a 7-days' growth on nutrient agar + 2 % glycerine suspended in 5 ml. sterile distilled water.

After 2½ months the following four distinct types of symptoms were observed in the experimental plants. With the exception of the fourth, these have already been described from runner plants of cultivated strawberries (CROSSE & PITCHER, 1952).

1. Proliferation of secondary crowns
2. Alaminate leaves (Fig. 2)
3. Cauliflowers and near-cauliflowers (Fig. 3)
4. Leafy galls (Fig. 4)

The leafy galls occurred exclusively in plants inoculated with the bacterium only. They resembled cauliflowers in general appearance but differed in certain particulars. Thus, where leaves were recognisable as such, they were smaller than normal, but unlike cauliflower, the petioles were not thickened and recurved, and the laminae not reduced in size relative to the petioles. Further, in leafy galls the laminae were more or less membraneous and petioles whitish and translucent, compared with the coarser laminae and brownish opaque appearance of cauliflowers. Finally there was generally much more undifferentiated gall tissue in the leafy galls than in cauliflower. The differences between these two symptoms will be seen by comparing Figs. 3 and 4. The final distribution of symptoms under the four treatments is shown in Table I.

TABLE I

Inoculations of F. vesca seedlings with A. ritzema-bosi and/or C. fascians (10 plants/treatment)

Inoculation	No. of plants bearing: Alaminate leaves	Cauliflowers	Leafy galls	Total no. of secondary crowns per treatment
Eelworm	8	0	0	9
Eelworm + bacterium	7	8	0	28
Bacterium	0	0	8	72
None	0	0	0	0

Fig. 1. *Fragaria vesca* seedling raised on autoclaved soil.

R. S. PITCHER & J. E. CROSSE: *Relationship of eelworms and bacteria to certain plant diseases, II.*

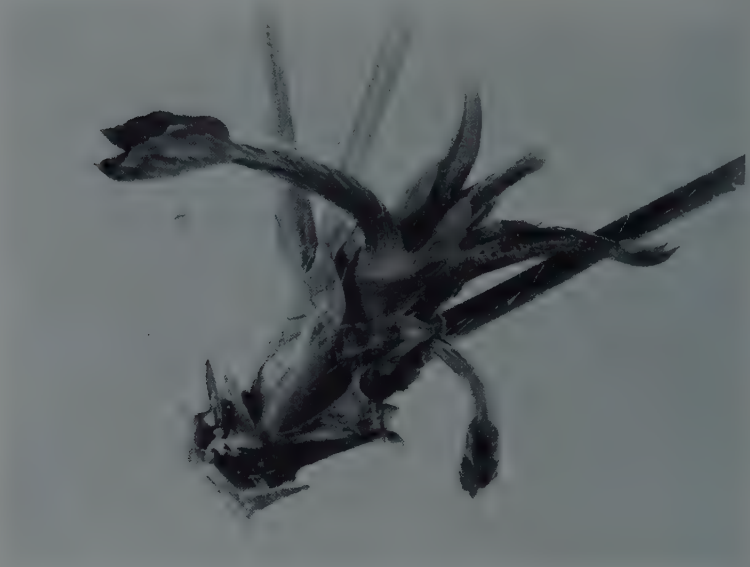

Fig. 2. Alaminate leaves on *Fragaria vesca* seedling inoculated with *Aphelenchoides ritzema-bosi.*

Fig. 3. Cauliflower on *Fragaria vesca* seedling inoculated with *Aphelenchoides ritzema-bosi* and *Corynebacterium fascians.*

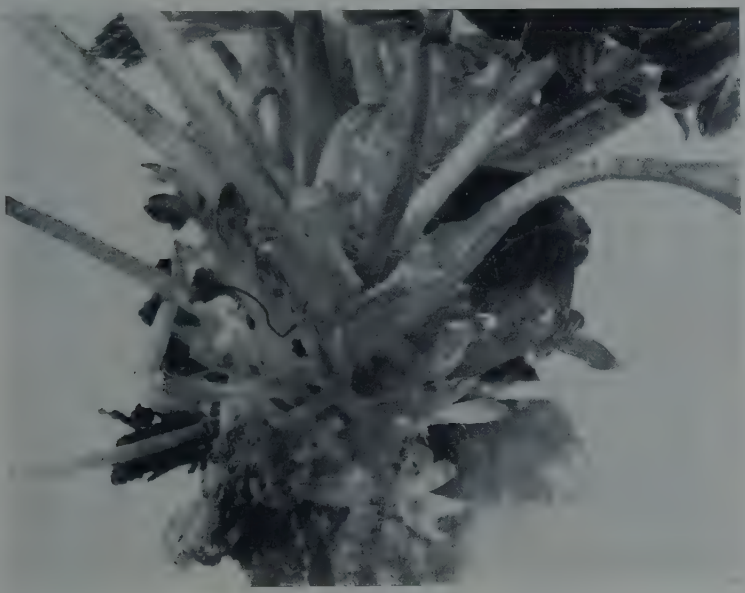

Fig. 4. Leafy gall on *Fragaria vesca* seedling inoculated with *Corynebacterium fascians.*

Fig. 5. *Fragaria vesca* seedling raised aseptically on an inorganic nutrient agar medium.

As in previous experiments with potted runner plants, cauliflowers developed only where eelworms and bacteria were co-inoculated. The occurrence of leafy galls in 8 of the plants inoculated with *C. fascians* alone was, however, in complete contrast to the consistently negative response of runner plants. LACEY (1942) observed a similar difference in the susceptibility of seedling and runner plants to *C. fascians*. Probably the leafy galls on strawberry seedlings are comparable to those caused by *C. fascians* on other plants and cauliflowers are simply leafy galls modified by the activities of eelworms. In addition, the eelworms inhibited secondary crown production by the bacterium, which fell from 72 to 28 crowns per treatment ($P < 0.05$) when the eelworm was co-inoculated.

Characteristic alaminate leaves were not produced by *C. fascians* alone, but as in previous experiments with runner plants, they appeared consistently wherever the eelworm was inoculated. However, this further evidence that eelworms were the sole cause of alaminate leaves could not be considered conclusive because bacterial contaminants, including an organism resembling *C. fascians*, were subsequently demonstrated in all jars of all treatments. Two of those isolated were positively identified as *C. fascians* by their pathogenicity to sweet peas.

Although this experiment failed in its primary object of determining the effect on strawberry of eelworms alone, it threw fresh light on the relative roles of eelworm and bacterium in the etiology of cauliflower disease by showing (1) that on seedling strawberries the bacterium alone could cause leafy galls, but not cauliflower; (2) that the eelworm was not merely a vector, but was directly responsible for part of the cauliflower symptom.

Experiment 2. The high degree of contamination in the uninoculated control plants of Experiment 1 suggested that bacteria had been introduced with the seed, or, more probably, that they had gained entry to the jars during the 5-6 months these had stood in the greenhouse. For these reasons the following modifications were now introduced.

1) seeds were sterilised in mercuric chloride and washed under reduced pressure in a closed circuit apparatus, and also separated on a sterile absorbent paper for germination 2) seedlings were raised in simpler containers consisting of half-pint milk bottles with the neck plugged with cotton wool and covered by an inverted plastic egg-cup. Apart from these and the fact that eelworms were obtained from "wool" on strawberry leaves the procedure was the same as in Experiment 1.

Symptom expression followed the same pattern as before, but

probably because of the more rapid plant growth in the prevailing summer conditions, symptoms developed much less consistently. All the eelworm inoculations were successful, numbers increased from about 25 per plant to over 1000 in many cases.

At the conclusion of the experiment contaminants were observed in all plants, with the exception of one uninoculated control. The degree of contamination was less than in Experiment 1; in one instance it was limited to a single bacterial species and despite careful tests *C. fascians* was not demonstrated in any plant. This gave the first clear indication that this bacterium was not directly concerned in the formation of alaminate leaves, but because of the difficulty of isolating this organism in the presence of more rapidly growing saprophytic bacteria, the failure to detect *C. fascians* was not accepted as conclusive evidence of its absence.

SEEDLINGS GROWN ON AGAR MEDIUM

These experiments concentrated primarily on the problem of establishing pure cultures of *A. ritzema-bosi* on strawberry seedlings. The following modifications were now introduced to reduce the chances of incidental contamination during the various stages of experimental procedure:

1. The large containers with a soil medium, originally chosen because they appeared to offer the best and simplest means of obtaining good plant growth, were replaced by hard glass tubes (3 cm × 15 cm) containing an inorganic nutrient agar medium (Fig. 5).
2. Manipulative procedures during eelworm sterilisation were reduced.
3. A rapid method of eelworm inoculation was adopted.

Growth of Seedlings. The nutrient medium had the following composition per litre: 4.14 ml M.$NaNO_3$, 1.0 ml M.KH_2PO_4, 1.0 ml M.K_2SO_4, 2.0 ml M.$MgSO_4$, 4.5 ml M.$CaCl_2$, 5.08 ml M.$NaSO_4$, 1.0 ml of trace element solution containing Zn, Cu, Bo, Mn, and 0.05 gms $FeSO_4$, $7H_2O$. The pH was adjusted to approximately 6.2. The 1 % agar was melted and 20 ml of the medium added to each tube and these then sterilised at 15 lbs/20 mins. The agar was allowed to set with the surface at 45° to the wall of the tube and 5 ml sterile distilled water then added. The final pH of the medium was approximately 5.6.

Seeds were soaked overnight and then sterilised for 20 minutes in chloramine-T under reduced pressure. After washing three times, the seeds were transferred to the agar tubes with a copper spoon beaten from the end of thick gauge copper wire. The plugs were then covered

with plastic caps and the tubes stood in the window of the laboratory. Growth of the seedlings on the agar medium was good, except for a slight tendency towards blackening of the roots.

The agar tube method had three main advantages over the soil bottle method, namely 1) the necks of the tubes could be adequately flamed when the plugs were removed, 2) seeds were much more easily and quickly deposited on to the agar than into soil, 3) contaminations occurring before inoculation could usually be recognised and the tubes discarded.

Sterilisation of eelworms and inoculation of seedlings. Eelworms were extracted from chrysanthemum leaves and sufficient inoculum for each plant was treated in a separate sterile 3 ml centrifuge tube and not bulked in a petri dish as hitherto. Tubes were plugged with cotton wool, covered with an aluminium cap and to each was added 1 ml of a suspension of approximately 250 eelworms in 0.02 % dioctyl sodium sulphosuccinate, followed by 1 ml of 7.5 p.p.m. ethoxy ethyl mercury chloride solution. The final concentrations of wetter and sterilant were thus the same as those previously employed. The eelworms were left in the sterilising solution for 96 hours, washed once, and finally resuspended in 0.75 ml of sterile distilled water. Inoculations were made by quickly pouring the contents of one centrifuge tube over each plant. All operations requiring removal of the plug from the agar tubes were carried out under a hood.

Experiment 3. Seeds were sown in mid-June and the seedlings inoculated in early December. Six replicate plants per treatment were inoculated with (a) eelworms, (b) *C. fascians,* (c) eelworms + *C. fascians.* The uninoculated controls received 0.75 ml of sterile distilled water. Bacterial inoculum was added from a pipette and the co-inoculations made by adding a small drop of bacterial suspension to the eelworm inoculum in the centrifuge tubes immediately before this was poured over the plants.

Disease symptoms were recorded at the beginning of March. All six plants inoculated with eelworms alone, and four co-inoculated with eelworm + bacterium, bore characteristic alaminate leaves; three of the co-inoculated plants had cauliflowers and the other three abnormalities suggesting incipient cauliflowers. Only one of the plants receiving the bacterium alone had developed a leafy gall by the time records were made. All control plants were normal.

The uninoculated plants and plants inoculated with eelworms only

were tested for contaminants as follows: Ten ml of sterile distilled water, sufficient to cover the crown of the plants and base of the petioles, was poured into each tube. Tubes were allowed to stand for 3-4 hours with intermittent agitation to dislodge any superficial contaminants from the plant or agar, and the water then decanted into empty sterile tubes. From two eelworm inoculated plants and one control plant the water was distinctly turbid, indicating a high degree of contamination; from the remaining plants the water was clear. Next, 1 ml aliquots of the water from each plant were mixed in a plate with Difco nutrient agar containing 1 % mannitol and 1 % glucose; the plates were incubated at 25° C for 14 days and examined periodically for growth. Where contamination was already evident, as indicated by turbidity, dilution plates were poured to obtain estimates of the numbers of micro-organisms present. The results for the control and eelworm-inoculated plants are shown in Table II. It will be seen that plants were either contaminated heavily or not at all. All plants inoculated with eelworms bore alaminate leaves but only one was contaminated with bacteria.

TABLE II

Distribution of alaminate leaves and contaminant micro-organisms in uninoculated and eelworm-inoculated seedlings of F. vesca.

	Eelworm-inoculated						Uninoculated					
Replicate No.	1	2	3	4	5	6	1	2	3	4	5	6
No. alaminate leaves/plant	3	4	2	2	3	1	0	0	0	0	0	0
Bacteria	+[1]	—	—	—	—	—	—	—	—	—	—	+[3]
Fungi	+[1]	—	+[2]	—	—	—	—	—	—	—	—	+[3]

+ present — absent

Notes. 1. A *Cladosporium* spp. and 2 gram negative bacteria. Total no. bacteria 70×10^6
2. A pink yeast. Total no. 10^6
3. *Pullularia pullulans* and a gram negative bacterium.

Experiment 4. This experiment resembled the previous one, but was restricted to a comparison between plants inoculated with eelworms (12 plants) and uninoculated controls (6 plants). Seeds were sown in March, the seedlings inoculated in July and the results assessed in October.

Plants were tested for contaminants by the same procedure as before except that after the plants were covered with sterile distilled water

they were allowed to stand overnight before isolation plates were prepared. After 6 days all plants judged free from contaminants by the plate test were tested more stringently by detaching a single alaminate leaf from each plant, cutting it into 3 pieces and transferring each piece separately to a tube of nutrient-mannitol-glucose broth. After 2 weeks incubation at 25° C and a further 2 weeks at room temperature no growth occurred in any tube, thus confirming the results of the plate tests. When the broth was subsequently inoculated with *C. fascians* heavy growth was obtained, indicating, that the absence of growth in the first place was not due to inhibitory substances carried over with the plant material. At the conclusion of this and the previous experiment many active eelworms were observed in the free water in the tubes and endoparasitic eelworms, larvae and eggs were demonstrated in the leaves of the plants, indicating that pre-inoculation treatments had not impaired their invasive or reproductive capacity.

Table III shows the results for the 12 plants inoculated with eelworms. All bore alaminate leaves; 7 were completely free of contaminants and 8 free of bacterial contaminants; *C. fascians* was not present in any of these plants. All the control plants were normal and none was contaminated.

TABLE III

Distribution of alaminate leaves and contaminant micro-organisms in F. vesca seedlings inoculated with eelworms

Replicate No.	1	2	3	4	5	6	7	8	9	10	11	12
Alaminate leaves	+	+	+	+	+	+	+	+	+	+	+	+
Bacteria	—	—	—	—	+	+	—	—	+	+	—	—
Fungi	—	+	—	—	—	—	—	—	—	—	—	—

+ present — absent

The results of this and the previous experiment show conclusively that the eelworms can cause alaminate leaves in the absence of all other micro-organisms. It follows therefore that *C. fascians* plays no direct part in the etiology of this symptom. In both experiments the majority of the plants inoculated with eelworms were moribund by the time the final records were made. By contrast the control plants and those inoculated with *C. fascians* alone were healthy. Of the two etiological agents, therefore, the eelworm was clearly the more lethal to strawberry seedlings.

DISCUSSION

In the light of the above it is necessary to modify certain tentative conclusions drawn from previous experiments with runner plants (Crosse & Pitcher, 1952). The fact that *C. fascians* is commonly associated with alaminate leaves in field strawberries had lead us to think that it might be causally implicated. The experiments with seedlings make it clear, however, that alaminate leaves are due solely to eelworms acting as primary plant parasites. It seems probable also, judging by their lethal effects on seedling strawberry plants, that the eelworms are responsible for the killing of crowns and the formation of open-centre plants which usually follow the development of alaminate leaves in field strawberries.

The experiments with seedlings confirm the dual role of eelworms and bacteria in cauliflower disease, but the differences between cauliflower and bacteria-induced leafy galls show that the eelworms are more actively involved in the symptomatology of the former than was at first thought. Thus the thickening and recurving of petioles and suppression of leaf laminae which chiefly distinguish cauliflower from leafy galls can be clearly attributed to the eelworms, as they are also characteristic of alaminate leaves, a symptom entirely of eelworm origin. The eelworm also appears to check the development of secondary crowns and gall tissue, the stimulus for which is provided by the bacterium. A cauliflower may thus be considered primarily as a hyperplastic bacterial symptom, modified in detail by the activities of the eelworm, which, in all but small seedling strawberries, is also essential as a vector of the bacterium. True leafy galls comparable to those on the experimental seedlings have never been observed in the field, where, in our experience, hyperplastic disturbances of runner plants appear only in the presence of eelworms and are invariably of the cauliflower type.

From these investigations it is concluded that two related but distinct diseases may be found in eelworm-infested field strawberries, namely:

1. A true *eelworm* disease, resulting in feeding-areas, alaminate leaves, and, following the death of these structures, open-centre plants. *C. fascians* is associated with alaminate leaves but plays no direct causal role.

2. A predominantly *bacterial* disease, cauliflower, composed of a leafy gall initiated by *C. fascians* and modified by the eelworms. It occurs in only a few infested plants and is often accompanied by true

eelworm symptoms, *e.g.*, feeding areas and alaminate leaves.

The low incidence of cauliflower disease in infested strawberries, despite the invariable presence of *C. fascians*, presents an outstanding problem. One probable reason may be the comparative rarity of strains of the bacterium capable of inducing hyperplastic disturbances in strawberry plants. Variability and host specificity are characteristic of this organism and are known to occur amongst strawberry isolates (Lacey, 1934; Jacobs & Mohanty, 1951; Crosse & Pitcher, 1952). Factors other than specificity may also be involved, however, for as Jacobs & Mohanty (1951) have pointed out, 100 % infection with *C. fascians* is rarely obtained even under aseptic conditions, using hosts of known suceptibility and bacterial strains of known virulence. The precise nature of these factors has not been determined, but it seems fairly certain they must operate in the field as well as in the laboratory.

Comparatively little is known of the ecology of *C. fascians*. Organisms closely resembling it have been isolated from healthy strawberries and other plants, but as yet none has been shown to be pathogenic (Crosse & Pitcher, 1952; Lacey, 1955). The organism is undoubtedly widespread and we are inclined to the view that it is normally present in healthy strawberries as a saprophyte which may become pathogenic when the plants are invaded by eelworms. Evidence of its independent saprophytic existence has been obtained from a recent field experiment in which we found that a pathogenic strain of *C. fascians* survived in field strawberries in the absence of eelworms for at least six months, without causing disease symptoms and without any apparent decline or loss of virulence. There is no doubt, however, that a fuller understanding of cauliflower and related diseases is dependent on further ecological research.

ZUSAMMENFASSUNG

Weitere Untersuchungen über die Ätiologie der Blumenkohlkrankheit der Erdbeere wurden mit aseptisch angezogenen *Fragaria vesca*-Sämlingen durchgeführt, die dann mit Reinkulturen von *Aphelenchoides ritzema-bosi* und selektierten Stämmen von *Corynebacterium fascians* einzeln oder kombiniert beimpft wurden. Frühere Schlussfolgerungen, dass die Blumenkohlkrankheit komplexer Natur ist, die die Anwesenheit beider pathogener Organismen erfordert, wurden bestätigt. Es konnte gezeigt werden, dass das Älchen zum Krankheitsbild aktiv beiträgt.

An mit Älchen beimpfte Erdbeersämlingen wurden Blätter mit reduzierten Blattflächen in Abwesenheit aller sonstigen Organismen hervorgebracht. Es wird daraus geschlossen, dass Blätter mit reduzierten Blattflächen, die unter Feldbedingungen auftreten, in erster Linie dem Älchen zuzuschreiben sind, obwohl sie gewöhnlich schwer mit *C. fascians* besetzt sind. Blattgallen wurden experi-

mentell an Sämlingen durch Einimpfen ausgewählter Stämme von *C. fascians* hervorgerufen, aber es ist zweifelhaft, ob echte Blattgallen überhaupt unter Feldbedingungen auftreten. Die Ökologie von *C. fascians* und ihr Verhalten zur Symptomatologie der Blumenkohlkrankheit werden diskutiert.

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Received for publication: February 27, 1958

Nematologica III (1958) : 257-268. E. J. Brill, Leiden

PEA ROOT EELWORM IN THE EASTERN COUNTIES OF ENGLAND

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Pea root eelworm, *Heterodera göttingiana* Liebscher, was first recognised in the Eastern Counties in 1922 and was recorded on a further nine fields only between this date and 1946. However, between 1948 and 1956, coinciding with the increase in the pea acreage after the war and with greater advisory activity due to the commencement of the National Agricultural Advisory Service, a total of 47 infested fields was discovered. These attacks were mainly on peas (*Pisum sativum* L.) although occasionally the eelworm was found on field beans (*Vicia faba* L.).

The object of this paper is to give the present position in the Eastern Counties, to present evidence on the importance of field beans as a host and to explain pea failures on fields which have not grown many host crops during the years immediately preceding the attacks.

The map (Fig. 1) shows the distribution of pea root eelworm infestations in the Eastern Counties. There is at least one known infested field in every county and one area, namely in East Suffolk, where the eelworm occurs more commonly than elsewhere. Some of the fields, particularly in Essex, have grown peas at least once every three years and these infestations have almost certainly been built up and maintained by this practice. Many other fields, particularly in East Suffolk, on which severe attacks have occurred have not grown many host crops during recent years although they may have done so many years ago. As there is a concentration of infested fields in East Suffolk this county was selected for further study in an attempt to find an explanation for this.

On many of the infested fields in East Suffolk the pea crop failed completely. On others the crop was patchy but as the season advanced the patches enlarged and further ones appeared giving rise to farmers' fears that the eelworm was spreading rapidly. This symptom probably

occurs to some degree with most *Heterodera* species but it is very spectacular with *H. göttingiana* and suggests that the pea plant is very susceptible.

Field observations have suggested that field beans are far less sus-

Fig. 1. Distribution of pea root eelworm in the Eastern Counties of England.

ceptible than peas to attacks by pea root eelworm. Walton, Ogilvie & Mulligan (1933) found broad beans infested in four localities in 1933. There was little effect on the growth of the plants although sometimes the lower leaves were slightly yellow and the plants somewhat dwarfed. In East Suffolk three crops of field beans affected by pea

root eelworm have recently been encountered. In two fields patches of stunted plants associated with eelworm were present and the other crop was a failure. Two of these crops were in adjoining fields which had been treated and cropped similarly in the past, the main difference being that one field had grown beans once every four years during the previous 23 years and the other had often grown root crops rather than beans. The beans on the former field were a failure and on the latter fairly good, except for one patch which was said to coincide with the position of a patch in a pea crop grown eight years previously. The eelworm populations in these two fields in the following autumn were 120 and 43 eggs/g respectively.

In the Eastern Counties pea root eelworm attacks occur on both heavy and light soils as was also found by WALTON *et al* in the S.W. of the country. In East Suffolk many of the infestations in the middle of the county are on heavy clay soils whereas those on the coastal strip are generally on sandy loam soils. This is in marked contrast to the other common *Heterodera* species which in England are mainly confined to the lighter soils.

In 1948 a host range trial was carried out in pots and on an infested field in East Suffolk. This confirmed previous workers' results that the only commonly-grown crop hosts were peas, field and broad beans (*Vicia faba*), and vetches (*Vicia sativa, Vicia villosa*). Later many species of weeds were examined on a number of heavily-infested fields but no cysts could be found on any of them. Wild vetches (*Vicia spp*) occur mainly as verge and hedgerow plants and have not been seen as weeds on infested fields.

Many of the fields in East Suffolk on which pea root eelworm failures have occurred, had not grown host plants very frequently during the years immediately preceding the attacks. The cropping histories have been obtained for as many of these fields as possible. In many instances the farmer has been able to give a good idea of the number of host crops he has grown and occasionally it was the first pea crop grown during his occupation of the farm, sometimes going back 20-30 years, which failed and gave the first indication of the presence of the eelworm. For two fields (A and B) well-authenticated cropping histories are available and are quoted in full (Table I). For the remainder, the information is much less complete and this is presented in tabular form (Table II). Fields A and B, show clear evidence that few host crops had been grown during the periods quoted. Field A had a break of 12 years between host plants and field B one of 10 years. It is pos-

TABLE I

Cropping histories of two fields on which peas failed from eelworm attack.

Field A

1935 Barley	1941 Barley	1948 Barley
1936 Peas*	1942 Wheat	1949 Beans*
1937 Wheat	1943 Trefoil	1950 Wheat
1938 Barley	1944 Wheat	1951 Sugar beet
1939 Trefoil	1945 Barley	1952 Barley
1940 Wheat	1946 Sugar beet	1953 Peas*(failed)
	1947 Barley	

Field B

1929 Clover	1938 Turnips	1947 Wheat
1930 Wheat	1939 Sugar beet	1948 Sugar beet
1931 Oats	1940 Barley	1949 Barley
1932 Turnips	1941 Clover	1950 Peas*
1933 Barley	1942 Wheat	1951 Wheat
1934 Beans*	1943 Sugar beet	1952 Barley
1935 Trefoil	1944 Oats	1953 Vetches*
1936 Wheat	1945 Beans*	1954 Barley
1937 Wheat	1946 Wheat	1955 Sugar beet
		1956 Peas* (patchy crop)

* — Host crop

sible but unlikely with field B that the eelworm could have been introduced about seven years previously and multiplied on the three subsequent host crops. In Table II there are three fields which had in all probability not grown a host crop for 9, 10 and 11 years respectively, the first of these intervals being previous to a complete failure and the other two before patchy crops of peas. STANILAND (in litt.) quotes a severe attack on peas in south-west England where no host had been grown for 15 years. For many of these fields in East Suffolk the severity of the attack on peas, the high eelworm populations, and the few recent host crops suggest that the eelworm populations had been built up to high levels many years ago. The extent to which host crops were grown in East Suffolk in the past was therefore investigated to see if the present distribution of the eelworm could be correlated with this.

The pea and bean acreages for East Suffolk from 1869-1956 are shown in Fig. 2. Compared with these, the acreage of vetches was low, varying from 4 to 6000 acres a year with an average of about 5000. Since 1935 the vetch acreage has fallen gradually until in 1955 it was below 1000 acres. Until about 1920 E. Suffolk grew a large acreage of

TABLE II

Cropping data for field attacks of pea root eelworm in East Suffolk

Year of attack	Crop damage to peas	Years since last host crop (Years since peas in brackets)		Last host crops	Previous host crops: Peas	Beans	Vetches	Cropping available since	Remarks
1945	Failure	?	(>20)	Vetches	0	0	1	1925	No damage on part of field which was an old orchard until recently.
1948	Failure	7	(>35)	Beans	0	3 or 4	0	c. 1914	
1948	Failure	2	(>16)	Beans	0	2	0	1932	
1948	Failure	4	(>20)	Beans	0	Few	0	1928	
1948	Failure	?	(?)	Beans					4-course rotation with beans included every 8th year
1950	Patchy	3	(>7)	Beans	0	1	0	1943	
1951	Failure	8	(8)	Peas	1	0	0	1943	
1953	Failure	9	(>10)	Beans	0	1	0	1943	
1953	Failure	?	(>20)	Beans	0	?	0	1933	Beans every 8th year
1954	Poor	6	(6)	Peas	1	2	0	1938	
1954	Poor	4	(>13)	Beans	0	2	0	1941	
1955	Patchy	4	(4)	Peas	1	0	0	1951	Peas patchy in 1951 but far worse now
1956	Patchy	11	(11)	Peas	1	1	0	1942	
1956	Patchy	10	(>20)	Beans				1936	
1956	(Beans) V. Poor	2	(2)	Peas	1	5	0	1933	

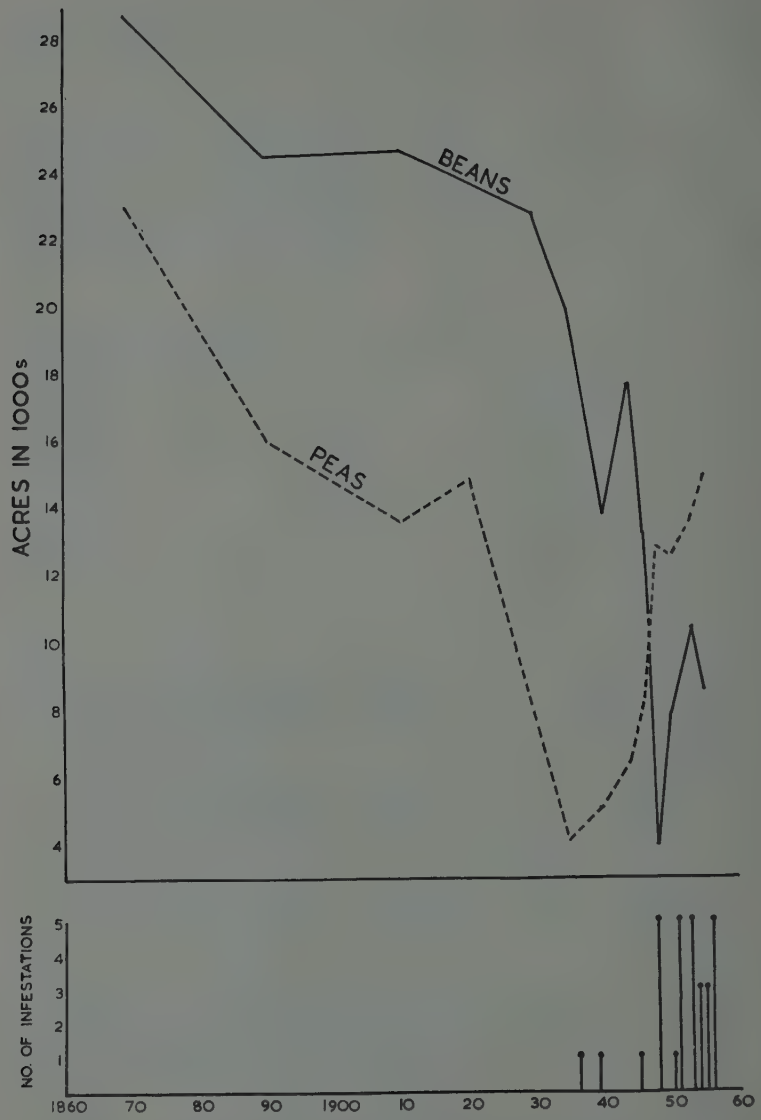

Fig. 2. Pea and bean acreages for East Suffolk and number of eelworm infestations.

host crops. In 1869, 18 % of the arable acreage [1]) in the county was under host crops, in 1890 15 %, and these figures would have been higher in the heavy land districts which grew a large acreage of beans. In 1940 and 1955 the figure was 7 % of the arable acreage.

Figure 2 shows that very few peas were grown in E. Suffolk during the 1930's, which is also true for the whole of England and Wales. The

Fig. 3. Parishes (shown in black) which grew peas on more than 8 % of their arable acreages. Pea root eelworm infestations are marked as black on white and *vice versa* (11 white and 19 black).

number of field attacks of pea root eelworm recorded in E. Suffolk is included and increases with the pea acreage from 1940 onwards.

The acreage figures for both peas and beans have been obtained for East Suffolk on a parish basis for three specimen years 1869, 1890 and 1910. The parishes which grew peas on more than 8 % of their arable acreages at least once in these three years are shown in Fig. 3. The pea acreage was therefore scattered fairly uniformly over the whole county

1) Arable acreage = all agricultural land except permanent grass.

irrespective of soil type. Fourteen parishes only grew peas on more than 12 % of their arable acreages at least once during these three years. Fig. 4 shows the parishes which grew the highest bean acreages and these are situated almost entirely in the heavy land districts of the county. These parishes grew beans on more than 12 % of their arable acreages at least once in the three years studied. When the pea and

Fig. 4. Parishes (shown in black) which grew field beans on more than 12 % of their arable areages. Pea root eelworm infestations are marked as black on white and *vice versa* (19 white and 11 black).

bean acreages were considered together it was found that, although beans accounted for by far the largest proportion of the acreage, most of these parishes and several others in the same area grew host plants on about 20% of their arable acreages. This means that the arable fields in these parishes grew either peas or beans on an average once in every five years and in fact many fields must have grown hosts on a considerably shorter rotation than this. The pea root eelworm distribution has been included in Figs. 3 and 4 and most of the records are seen to be in or near the bean-growing area.

A number of small (2-3 acre) fields have been soil sampled intensively each year following pea failures due to pea root eelworm. At each sampling fifty bulked cores were taken at random from each field, and

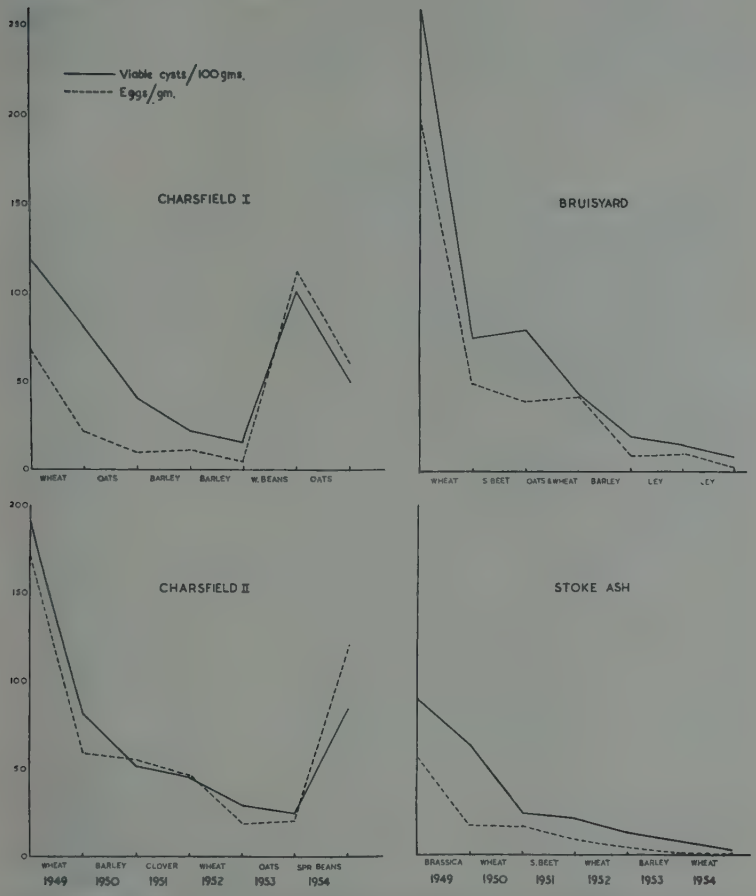

Fig. 5. Changes in pea root eelworm populations on four fields in East Suffolk.

200 g of air-dried soil were examined by the normal method in a Fenwick flotation apparatus (Goodey 1957). The graphs show a decline in the populations followed by increases when field beans were grown (Fig. 5). In one field which grew winter beans (Charsfield I) the population rose from 5 to 112 eggs/g and in another field (Charsfield II) an increase from 22 to 121 eggs/g was obtained with spring beans.

In a third field (not shown) the population two years before a spring bean crop was 9.6 eggs/g and afterwards had risen to 74 eggs/g. As winter beans have a much longer growing period than spring beans it is perhaps not unreasonable to expect that they would cause a greater population increase: in the above-mentioned field it was twenty-two times. In the two fields which grew spring beans the increase was about six to seven times.

Discussion

The annual rates of decay obtained from the graphs (Fig. 5) are approximately 40 % for 'viable cysts' and 45 % for eggs. These are similar to the accepted figures for potato root eelworm (*Heterodera rostochiensis*) and beet eelworm (*H. schachtii*) so that it does not appear that pea root eelworm is any more persistent than these two species.

The effect of peas and beans on the eelworm population has been investigated by Winslow (1955) and also by Jones & Moriarty (1956). Winslow working with infested soil from the East Suffolk coast obtained reductions in the eelworm populations when broad and field beans were grown in microplots and field plots respectively. These reductions occurred with a fairly high initial population of 127 eggs/g in the microplots and also with a low population of 15 eggs/g in the field plots. Jones & Moriarty working in microplots with the same infested soil to some extent confirmed the results of Winslow. They obtained very slight increases in three out of six microplots growing field beans, with initial populations varying from about 60 to 130 eggs/g and slight reductions in the other two. However, with an initial population of 10 eggs/g a four-times increase was obtained with field beans. Larger increases are normally to be expected with low initial populations than with higher ones approaching the "ceiling" value (Jones 1956). This is also evident in the garden plot experiment of Jones & Moriarty. In this experiment, with the lower range of initial populations varying from 9-91 eggs/g peas increased the population from 2 to 26 times and field beans from 1 to 19 times. With an initial population of 9 eggs/g field beans increased the population 19 times and with 13 eggs/g they increased it 5 times. At the higher range of initial populations varying from 112 to 218 eggs/g it is clear that the ceiling value has been approached for both peas and beans.

Many of the infested fields in East Suffolk have grown few recent host crops and Fig. 5 shows that high populations have decreased to a low level after a period of five to six years. The increases obtained

with field beans shown on these graphs must therefore be compared with the lowest initial populations quoted above and there is good general agreement. However the field plot result of WINSLOW in which field beans reduced the population from 15 to 11 eggs/g is anomalous. There is evidence that broad beans are less efficient hosts than field beans in the results obtained by JONES & MORIARTY. With "Longpod" and "Windsor" broad beans they obtained decreases in the eelworm population over an initial population range of 20-120 eggs/g.

JONES (1950) in attempting to explain the failure of peas on land which had not apparently grown host crops during recent years, suggested that the eelworm was extremely persistent, the pea plant was highly susceptible or there were unknown host plants. The work described here does not report the finding of unknown host plants, either crops or weeds, and does not support the view that the eelworm is any more persistent in the soil than some other *Heterodera* species. It does however suggest that the pea plant is very susceptible. A pea crop grown on an infested field acts as an indicator of the presence of the eelworm, and because of the devastating suddenness of the attack, advice may be sought whereas with the less-susceptible field beans attacks have perhaps usually passed unnoticed. There is good similarity between the present distribution of pea root eelworm in E. Suffolk and those parishes which grew the most beans during the latter half of the 19th and early 20th century, and also field beans, although they may not be as efficient hosts as peas, increase the eelworm populations under these conditions. It is therefore suggested that the explanation of these sporadic attacks on peas is that the eelworm populations were built up many years ago and, during the period when East Suffolk grew fewer host crops, were sufficiently maintained by the occasional field bean crop.

Pea root eelworm is not at present a serious pest in East Suffolk. However, the eelworm has been shown by survey to exist there in many fields and is therefore a potential danger particularly if the acreage of host crops is markedly increased or concentrated. The number of severe eelworm attacks increased with the acreage of peas from 1940 onwards. As the pea acreage rises still further there will be an increasing chance of finding more fields with long-established eelworm populations on which peas will fail.

ACKNOWLEDGEMENTS

Thanks are due to L. J. Coppock and N. D. N. Welford who did the

cyst and egg counts and who searched infested fields for weeds hosts of the eelworm. W. E. Dant and Miss M. Kiddy gave considerable assistance in the preparation of the illustrations for which I am very grateful.

Acknowledgement is also made to the Statistics Department of the Ministry of Agriculture for supplying the pea and bean acreages for the East Suffolk parishes.

ZUSAMMENFASSUNG

Heterodera göttingiana wurde auf 57 Feldern in den östlichen Grafschaften Englands angetroffen. In jeder Grafschaft gibt es wenigstens ein verseuchtes Feld; eine Massierung solcher Felder findet sich in der Grafschaft East Suffolk. Schwere Schäden an Erbsen und gelegentlich an Feldbohnen traten auf Lehmboden und auch auf leichteren Böden auf.

Erbsenverluste zeigten sich auf Feldern, die wenige neue Wirtspflanzen getragen haben. Die Fruchtfolge einzelner Felder wird mitgeteilt. Viele Befallsstellen treten in einem Gebiet von East Suffolk auf, das während der letzten Hälfte des 19. und eines Teils des 20. Jahrhunderts viel Bohnen (12% der bebauten Fläche) getragen hat. Der Anteil an Erbsen war während dieser Zeit ziemlich gleichmässig über die ganze Grafschaft verteilt. Die Erklärung für den sporadischen Befall an Erbsen dürfte darin zu suchen sein, dass sich die Älchenpopulationen viele Jahre vorher aufgebaut haben und während der Zeit etwa (1920-45), als in East Suffolk nur einige Wirtspflanzen angebaut wurden, sich durch den gelegentlichen Anbau von Feldbohnen halten konnten. Von einer Anzahl Felder wurden nach einem Erbsenschaden Bodenproben gesammelt. Die Diagramme zeigen ein Absinken in den Populationen, dem ein Ansteigen folgte, wenn Feldbohnen angebaut wurden. Die Ergebnisse werden mit den Befunden von Winslow (1955) und von Jones & Moriarty (1956) verglichen.

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Received for publication: January 15, 1958

Nematologica III (1958) : 269-273. E. J. Brill, Leiden

NEMATOLOGISCHE UNTERSUCHUNGEN AN HOPFEN. II. ZUR MORPHOLOGIE UND BIOLOGIE VON *HETERODERA HUMULI* FILIPJEV, 1934

VON

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Bei Untersuchungen über den Besatz von Hopfengärten mit Nematoden fand sich in fast allen untersuchten Gärten *Heterodera humuli* FILIPJEV, 1934 (SIMON 1957a, 1957b). Da die Art nach den vorliegenden Literaturangaben (VOIGT 1894, TRIFFIT 1929, DUFFIELD 1927, FILIPJEV & SCHUURMANS STEKHOVEN 1941, GOFFART 1951, FRANKLIN 1951) noch ungenügend bekannt ist, wurden einige Messungen an den Tieren durchgeführt. Zugleich sollte auch die Biologie des Tieres etwas beleuchtet werden. Noch während diese Arbeiten im Gange waren, kam die Ankündigung umfangreicherer Versuche über die Biologie von *H. humuli* durch GILLARD & VAN DEN BRANDE (1957). Es wurde daher von eingehenderen Untersuchungen abgesehen, um eine Überschneidung von gleichen Arbeiten zu vermeiden. Jedoch mögen die bisher erhaltenen Werte als Beitrag zur Kenntnis dieser Art mitgeteilt werden.

MORPHOLOGIE

Die Cysten von *Heterodera humuli* sind zitronenförmig und hell- bis dunkelbraun gefärbt. 100 Cysten wurden gemessen. Die Länge betrug 325,0-662,5 μ (489,8 μ), die Breite 212,5-462,5 μ (340,3 μ). a = 1,10-2,22 (1,46). Die Halslänge war bei 64 Cysten 25,0-100,0 μ (57,0 μ), die Halsbreite 37,5-62,5 μ (50,4 μ). Bei 36 Tieren war kein Hals vorhanden oder nicht deutlich zu erkennen. Die Vulva war bei 56 Cysten deutlich, bei 30 schwach und bei 14 überhaupt nicht vorstehend. Das Analfeld ist doppelt so lang wie breit, die Vulvabrücke kräftig, der Vulvaschlitz etwa so lang wie die Brücke. Ein Becken war nicht deutlich erkennbar. Die beiden Halbfenster sind etwa kreisrund, Bullae sind nicht vorhanden und der Anus liegt ziemlich in der Längsachse der Halbfenster. Die Cystenschale ist aussen fein getüpfelt, darunter ist ein unregelmässiges Zickzackmuster zu erkennen. Die Schale ist dünn.

Eine subkristalline Schicht war nicht zu erkennen. Form und Grösse der Cysten ist stark wechselnd (Abb. 1). Die meisten sind schwach gefüllt, nur wenige weisen mehr als 100 Eier auf. Leere Cysten sind auch unter neu gebildeten nicht selten.

In Wurzelnähe frei im Boden konnten keine Männchen beobachtet werden. Da sich aber in den Wurzeln einige Häutungsstadien fanden, konnten die Masse von FRANKLIN (1951) bestätigt werden. Die wenigen Tiere (3) entsprachen den von ihr gegebenen Werten.

Die Larven sind 327,2-407,2 μ (345,9 μ) lang und 18,2 μ breit. a

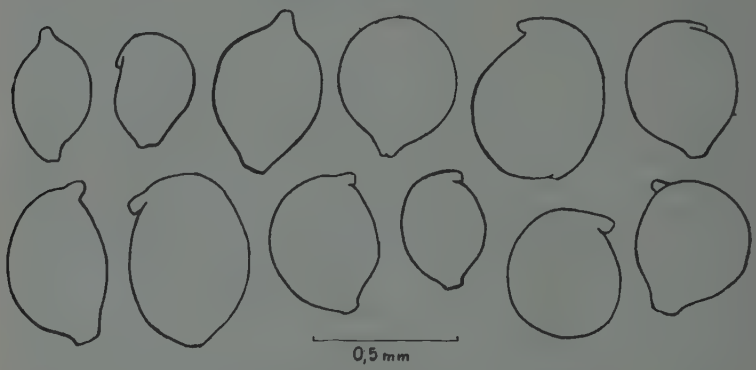

Abb. 1. Typische Cysten von *Heterodera humuli*.

beträgt 18,0-22,4 (19,6). Der Mundstachel hat eine Länge von 19,9-27,2 μ (22,1 μ) und ist am hinteren Ende deutlich geknöpft. Die Knöpfe sind leicht nach vorne gezogen. Die Cuticula ist deutlich geringelt, die einzelnen Ringe sind etwa 1 μ breit. Das Kopfgerüst ist deutlich, aber nicht stark ausgebildet Die Kopfkappe weist 3 undeutliche Ringe auf.

Die Eier sind 54,5-109,1 μ (94,7 μ) lang und 30,9-39,9 μ (36,6 μ) breit. a beträgt 1,8-3,3 (2,6).

Eine Zusammenstellung der Angaben der bisherigen Bearbeiter zeigt, dass die Art wohl stark variiert, jedoch in den wesentlichen Merkmalen gut übereinstimmt (Tabelle I).

BIOLOGIE

In den Bodenproben, die im Verlauf von Untersuchungen an freilebenden Nematoden genommen wurden, erschienen die Larven regelmässig und in ziemlich grosser Zahl im April und Mai; im Juni wurden sie seltener, waren aber auch noch im August und September zu finden.

Tabelle I

Masse von Heterodera humuli verschiedener Bearbeiter. Alle Masse sind in µ angegeben, die Durchschnittswerte stehen in Klammern.

Weibchen (Cysten)

	Länge	Breite	a	Halslänge
Voigt 1894	507,0 — 594,0 (564,0)	319,0 — 429,0 (377,0)	1,23 — 1,63 (1,44)	50,0 — 99,0
Duffield 1927	450,0 — 750,0	340,0 — 470,0		
Triffitt 1929	333,0 — 695,0 (534,0)	295,0 — 571,0 (402,0)	1,08 — 1,76 (1,36)	40,0 — 167,0 (70,0)
Hallertau 1957/58	325,0 — 662,5 (489,8)	212,5 — 462,5 (340,3)	1,10 — 2,22 (1,46)	25,0 — 100,0 (57,0)

Larven

	Länge	Breite	a	Mundstachel
Duffield 1927	380,0			
Triffitt 1929	316,0 — 480,0 (390,0)		23,0 — 29,0 (26,0)	23,0
Franklin 1951	399,7			
Gillard & van den Brande 1957	340,0 — 450,0 (395,0)			
Hallertau 1957/58	327,2 — 407,2 (345,9)	18,2	18,0 — 22,4 (19,6)	27,2 — 19,9 (22,1)

Eier

	Länge	Breite	a
Voigt 1894	93,0 — 113,0	39,0 — 51,0	2,0 — 2,4 (2,2)
Duffield 1927	92,0 — 95,0	47,0 — 48,0	2,0
Triffitt 1929	35,0 — 100,0	15,0 — 55,0	1,9 — 2,3 (2,1)
Franklin 1951	93,0	37,1	2.5
Hallertau 1957/58	54,5 — 109,1 (94,7)	30,9 — 39,9 (36,6)	1,8 — 3,3 (2,6)

Allerdings erscheinen sie dann nur noch sehr vereinzelt. Man wird also wie in England (DUFFIELD 1927) auch in unserem Bereich nur mit einer Generation rechnen können. Eine zweite Generation wäre nur unter sehr günstigen Witterungsbedingungen im Spätsommer und Herbst zu erwarten.

Die Stadien in der Wurzel konnten von verschiedenen Wurzelproben beobachtet werden. In der Form entsprechen sie den von anderen *Heterodera*-Arten beschriebenen sehr gut. Im ganzen sind die Tiere kleiner, lediglich die Schwanzspitze scheint deutlicher ausgebildet zu sein. Das untersuchte Material musste leider formalinfixiert werden, deshalb ist eine genaue Beschreibung dieser Stadien, besonders der inneren Anatomie, nicht möglich. Es konnten auch 3 Männchen beobachtet werden. Sie trugen einen auffallend grossen Mundstachel (vgl. FRANKLIN 1951). Weisse Cysten erscheinen ab Juni bis in den Herbst hinein. Die ersten braunen, vollreifen Weibchen traten Ende Juli auf.

Die Stärke des Befalls in den Hopfengärten ist unterschiedlich. Allgemein bewegen sich die Werte um 100 Cysten je 100 g Erde, können jedoch in vereinzelten Fällen bis über 500 Cysten ansteigen. Merkwürdig ist, dass auch bei derart hohen Zahlen kein deutliches Schadbild im Freiland auftritt. In einem Topfversuch zeigte sich jedoch nach einer künstlichen Infektion mit einer Ei-Larven-Suspension an Hopfensämlingen ein leichtes Welken der Blätter vom Rande her. In unbehandelten Töpfen trat die Erscheinung nicht auf. Man könnte daraus mit Vorsicht auf eine ähnlich toxische Wirkung der Suspension schliessen, wie sie VON DEN OUDEN (1956) für *H. schachtii* an Rübenblättern nachgewiesen werden konnte.

FUNDORTE UND GEOGRAPHISCHE VERBREITUNG

Heterodera humuli ist im Hopfenanbaugebiet Hallertau allgemein verbreitet. Wie Stichproben aus anderen Anbaugebieten zeigten, kommt die Art auch in Spalt, Hersbruck und Tettnang vor.

Wir kennen bisher die Art in Deutschland aus Sachsen (HOLLRUNG 1890), von Köln und Göttingen (VOIGT 1894). Ausserdem wurde *H. humuli* in folgenden Ländern Europas nachgewiesen: England (PERCIVAL 1895, DUFFIELD 1925, 1927, TRIFFITT 1929, PETHERBRIDGE & JONES 1944), Belgien (GILLARD & VAN DEN BRANDE 1957), Holland (OOSTENBRINK & DEN OUDEN 1953) und wahrscheinlich auch in Italien (GOFFART 1951). Ausserhalb des westlichen Europas sind Fundorte aus der UdSSR in der Ukraine (DMITRIEV 1954) und der Südafrikanischen Union (FILIPJEV 1934) bekannt.

SUMMARY

Heterodera humuli FILIPJEV, 1934, is regularly found in the hop centres of Hallertau, Spalt, Hersbruck and Tettnang (Southern Germany). Measurements of cysts, larvae and eggs are given and they agree well with those of other authors. The larvae appear in soil samples mainly in April and May, but continue to be found in a smaller number until September. The first ripe cysts are found towards the end of July.

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Eingegangen: 24. Februar, 1958

Nematologica III (1958) : 274-282. E. J. Brill, Leiden

HETERODERA MAJOR O. SCHMIDT, 1930 — POPULATION CHANGES IN THE FIELD AND IN POTS OF FALLOW SOIL

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POPULATION CHANGES IN FOUR FIELDS NEAR WINCHESTER

The soil here is shallow flinty loam overlying chalk; it is not very fertile and is prone to drying out. The fields on the farm are large (40-90 acres) and the main crops are spring cereals and seeds (linseed, mustard and grasses). Four fields were sampled for cereal root eelworm along fixed transects; two of the fields each had two transects so that medium and light soils were included. A trowelful of soil was taken about every ten yards along the transect, and the bulk sample was mixed, dried, and the eelworm population estimated, using techniques described elsewhere (GOODEY 1957). Sampling began in 1953, and continued annually; the cropping of the field was decided by the farmer. The changes in the eelworm population are shown in Table I.

Barley generally produced an increase in the number of larvae per gram of soil, the largest increase being about five times. Under Italian ryegrass and cocksfoot [1]), (both hosts of this eelworm) the eelworm population fell, as it did under fallow or a non-host crop, the reduction being about 60 %.

POPULATIONS ON FIELDS IN THE WEST MIDLANDS

In 1954 several fields in this area were sampled for cereal-root eelworm. Crop failure or severe damage had occurred to oats on most of these fields at some known earlier date, and their cropping history after this time was known. The soil on these fields was generally a light sandy loam of Triassic origin, with no chalk. Table II shows the results of these investigations.

The highest populations were found on the field sampled one year after an oat crop, all other populations were less than five larvae per gram of soil. As the size of the eelworm population after the damaged oat crop was unknown, it was impossible to estimate the amount or

1) See glossary of the English, German and Latin names of plants mentioned at the end of this paper.

TABLE I

Eelworm population and cropping on infested fields near Winchester

O.S. FIELD NO		FEB. 1953	CROP 1953	MAR. 1954	CROP 1954	MAR. 1955	CROP 1955	MAR. 1956	CROP 1956	MAR. 1957
	c.g.	0.65	Fallow	0.47	"Carlsberg II"	0.26	"Carlsberg II"	0.38		0.185
F71	la.c.	2.4	followed by Kale &	0.5	Barley	2.3	Barley	2.8	Barley	10.2
	la.g.	1.6	Ryegrass, sheeped in autumn ploughed in Jan. 1954	0.24		0.6	U/S It. Ryegrass (Killed by winter)	1.0		1.8
	c.g.	1.89		2.06	"Carlsberg II"	0.68		1.65	Cocksfoot	1.03
F73	la.c.	27.5	Cocksfoot	5.4	Barley	35.4	Barley	22.0	ploughed in in	11.0
	la.g.	51.9		11.1		23.8		36.3	July, followed by mustard.	11.3
	c.g.	0.24		0.16	Bastard Fallow	0.16		0.13		0.13
F78(A)	la.c.	34.0	Linseed	13.2	followed by Rape	8.9	It. Ryegrass	5.0	Barley	19.2
	la.g.	8.2		2.1	U/S It. Ryegrass	1.3		0.6		2.6
	c.g.	0.22		0.15	Bastard Fallow	0.08		0.09		0.125
F78(B)	la.c.	56.0	Linseed	24.0	followed by Rape	20.0	It. Ryegrass	4.1	Barley	15.0
	la.g.	12.6		3.6	U/S It. Ryegrass	1.5		0.4		1.8
	c.g.	0.30		0.32		0.17		0.45	Mustard	0.17
F92A	la.c.	13.0	Barley	65.8	Black Mustard	80.5	Barley	30.1	U/S Cocksfoot	22.3
	la.g.	3.9		21.0		13.9		13.7		3.8
	c.g.	0.49		0.78		0.56		1.13	Mustard	0.78
F92B	la.c.	29.7	Barley	96.0	Black Mustard	55.3	Barley	63.5	U/S Cocksfoot	16.6
	la.g.	14.6		74.9		30.7		71.9		12.9

c.g. = cysts per gram soil; la.c. = larvae per cyst; la.g. = larvae per gram soil; U/S = undersown.

TABLE II

Eelworm population in 1954 on fields in W. Midlands

Year and Crop														Cyst gram	Larvae cyst	Larvae gram	Field
1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954				
												Oats	Ley	.41	39.2	16	1A
												Oats	Ley	.07	228.8	16	1B
											Oats	Sugar-Beet	Ley	.13	22.7	3	2
										Oats	Clover	Lucerne	Lucerne	.15	12.4	1.9	3
									Oats	Clover-Ley	Clover-Ley	Clover-Ley	Clover-Ley	.23	10.9	2.5	4
									Oats	Mixed Corn	Ryegrass, Kale	Mixed Corn	Ryegrass, Forage Oat	.13	38.5	4.9	5
								Oats	Clover-Ley	Clover-Ley	Clover-Ley	Clover-Ley	Potato	.25	6.2	1.5	6
							Oats	Cocksfoot	Cocksfoot	Cocksfoot	Cocksfoot	Cocksfoot	Cocksfoot	.05	2.3	0.1	7
Oats	Peas, Carrot	Kale, Beet, Mangold	Barley	Clover	Clover	Potato	Wheat	Swedes, Potato	Sugar-Beet	Barley	?	Potato	Mixed Roots, Kale	.07	17.3	1.2	8
Oats	Peas, Carrot	Kale, Beet, Mangold	Barley	Clover	Clover	Potato	Wheat	Swedes, Potato	Sugar-Beet	Barley	?	Swedes after Grazed oats	Mixed roots, Kale	.04	8.5	0.3	9
								Mixed corn	Potato	Mixed corn	Potato	Mixed corn	Ley	.1	38.5	3.8	10

Note: — damaged crops shown in italics.

rate of the population decline. If a high eelworm population at the commencement of the "damage year" is assumed, it is clear that the eelworm population fell under cocksfoot, leys, and with infrequent cereal cropping, but the pest was not eliminated.

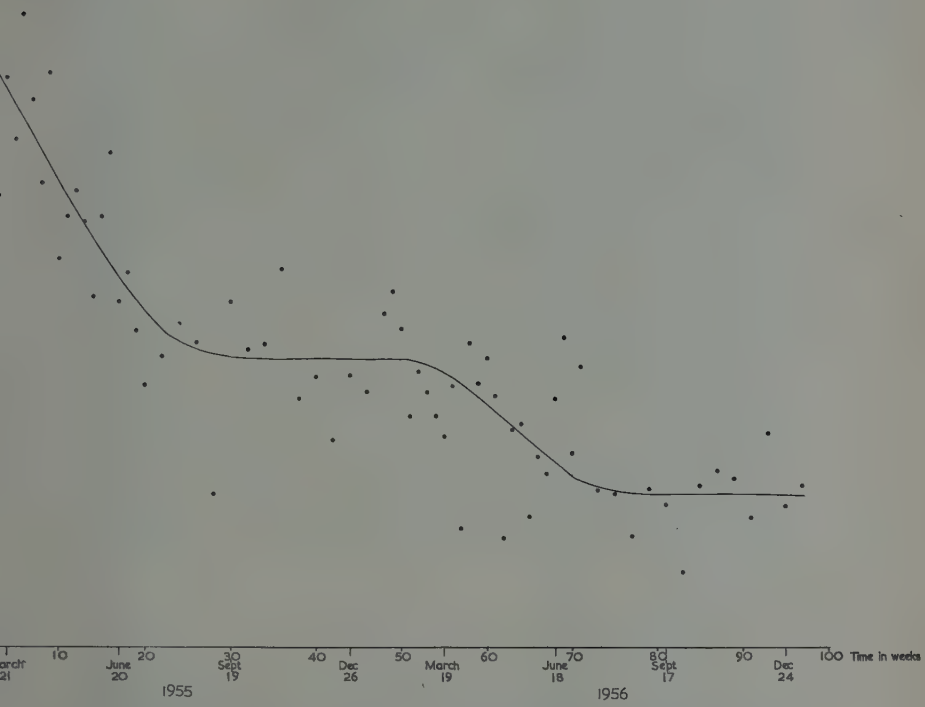

Fig. 1. Graph showing the decline in the number of larvae per cyst of *Heterodera major* over a period of almost two years. The estimations were made at weekly intervals in the spring and early summer and at fortnightly intervals during the rest of the year.

The heterogeneity of field populations and the errors introduced by the sampling and laboratory techniques limit the usefulness of the data obtained from the field investigations. For this reason it was decided to measure the fall of the eelworm population under relatively controlled conditions. In a preliminary experiment samples were taken at intervals from nine pots of well mixed infested soil. The population of *H. major* fell from 246 larvae/g to 83 larvae/g between 20th March

and 7th July, 1954. Between the latter date and 6th October, 1954 the population fell to 72 larvae/g. SCHMIDT (1930) showed that the peak of larval invasion of oat roots occurred in April and early May, and HESLING (1956) found that the emergence of the larvae from cysts kept in the laboratory lasted about ten days. These findings indicated the need for a continuous check on the population fall in soil, to discover whether the larvae have a mass-hatch of short duration in the spring. Accordingly, an experiment was set up in March 1955. Cysts of *H. major* were extracted from moist soil and selected for fullness and size by methods described elsewhere (HESLING 1956, 1957). About 380 g of sandy, "eelworm free", soil (PETERS 1951) and fifty of the selected cysts were mixed and added to a 3½ in. diameter plant pot. Eighty pots were so filled and placed at random in a gravel plunge. The pots were kept free of weeds and were watered in dry weather. The eelworm content of one pot was estimated [using methods described by GOODEY (1957)] at intervals of one week between March and July, and at intervals of two weeks during the rest of the year. Figs. 1, 2 and 3 show how the eelworm population fell over almost two years.

In January 1957 the contents of ten unexamined pots were mixed with more eelworm free soil, and each was transferred to a 6 in. pot. Six seeds of "Herta" barley were sown in each pot on 18th April. In mid July the soil mass in each pot was carefully turned out and examined for cysts, which were found on the roots of some of the plants. The soil was returned to each pot and in January 1958 the cysts in these pots were extracted and counted. The results are shown in Table III.

TABLE III

Number of cysts found after growing barley in uniformly infested soil after two years' fallow

Pot Number	Initial infestation (Mar. 1955) cysts/pot	eggs/pot	Number of cysts/pot Jan. 1958
14	50	22,400	766
66	"	"	232
20	"	"	142
77	"	"	47
25	"	"	35
5	"	"	136
17	"	"	463
39	"	"	246
31	"	"	42
32	"	"	577
			mean 268

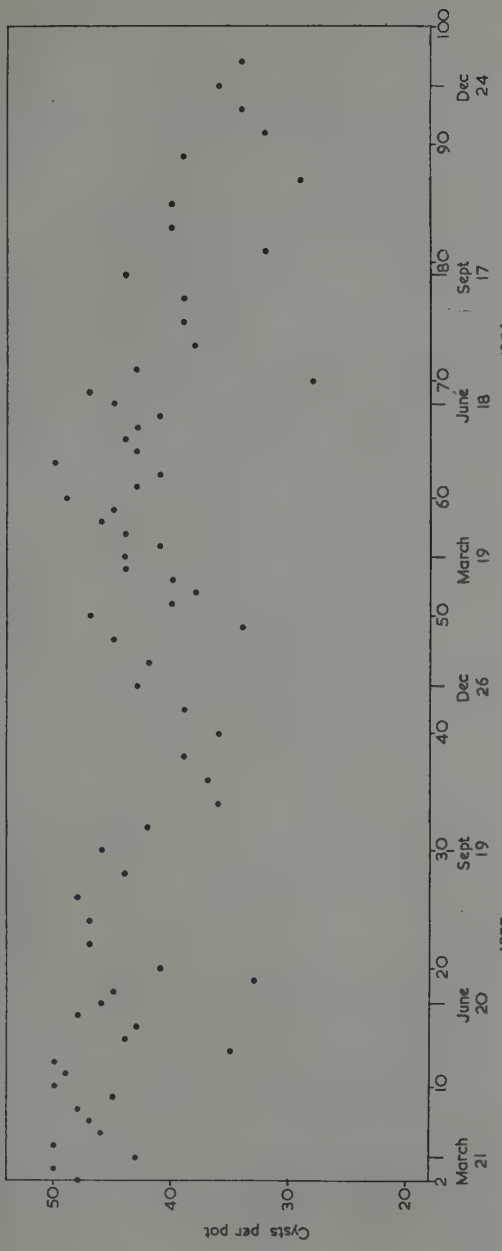

Fig. 2. Each point represents the number of *H. major* cysts recovered from a pot of fal'ow soil. At the start of the experiment each pot contained fifty large cysts.

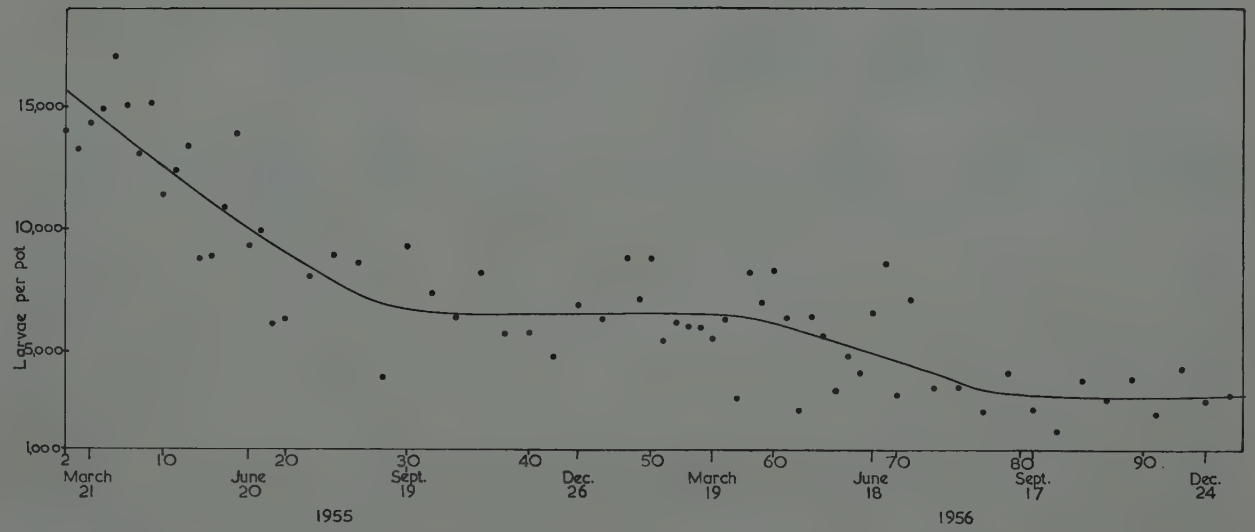

Fig. 3. Graph showing the decline in the number of eggs and/or larvae of *H. major* in pots of fallow soil. Each point represents the product of larvae/cyst and cysts/pot.

DISCUSSION

The increase of the cereal root eelworm in the field is not as high as that found in pot experiments (Hesling 1957). There is no doubt that barley is a host plant potentially capable of producing high field populations.

The population fall of *H. major* under fallow or non-host crop appears to be about 60 % in one year. Most of the larval emergence occurs between mid-March and mid-July, there is no evidence of a "mass-hatch" of short duration. The decline in the number of large new cysts in fallow soil in pots is slight, amounting to a loss of about 30 % in two years. In the field, where all sizes of cysts occur, and the soil is stirred by cultivation etc., the rate of loss of new cysts may be higher. In these respects, *H. major* resembles *H. schachtii*, for which species Jones (1956) estimated that the rates of decay of cysts and of eggs were 20 % and 50 % per annum, respectively. Wallace (1956) showed that there was a high rate of larval emergence from cysts of *H. schachtii* during April and May, and suggested that the low rate of emergence in the latter part of the summer was due to the high pressure deficiency of soil water at this time of the year.

The same reason may also explain the low emergence from cysts of *H. major* during this period, but cannot explain the absence of hatch in the winter. It is possible that other physical conditions (e.g. low temperature) may inhibit hatching at this time, or the eelworm may have a "dormant period" (Lownsbery 1951).

The writer wishes to thank Messrs. W. E. H. Hodson, P. R. J. McMorland, H. C. F. Newton and T. Hunt for their assistance and co-operation and Miss J. Daniels for help in the laboratory.

ZUSAMMENFASSUNG

Eine Felduntersuchung über die Veränderungen in der Population von *Heterodera major* ergab ein Absinken der Population nach Brache oder Nichtwirtspflanzen von etwa 60 % im Jahr bezogen auf Eier je g Boden. Gerste steigerte die Population auf das 5-fache, während Gräser als Nährpflanzen die Population verminderten. Sie scheinen also als Überträger nicht so gefährlich zu sein wie zuerst befürchtet wurde. Bei zweijährigen Topfversuchen mit Bracheboden trat in jedem Jahre ein Absinken der Population im Frühjahr und im Frühsommer ein und betrug jedesmal etwa 60 %. Die nach zwei Jahren übrig bleibende Population war imstande, grosse Mengen neuer Cysten zu entwickeln.

GLOSSARY OF PLANTS MENTIONED IN THE TABLES AND TEXT

Latin	*English*	*German*
Avena sativa Linn.	Oat	Hafer
Beta vulgaris Linn.	Sugar beet	Zuckerrübe
Beta vulgaris Linn.	Mangold	Mangold
Brassica napus Linn.	Swede	Kohlrübe
Brassica napus Linn. var *oleifera* D.C.	Rape	Ölraps
Brassica nigra Koch	Black mustard	Schwarzer Senf
Brassica oleracea Linn.	Kale	Kohl
Dactylis glomerata Linn.	Cocksfoot	Gemeines Knauelgras
Daucus carota Linn.	Carrot	Möhre
Hordeum vulgare Linn.	Barley	Gerste
Linum usitatissimum Linn.	Linseed	Lein
Lolium multiflorum var *italicum* Lam.	Italian rye-grass	Ital. Raigras
Medicago sativa Linn.	Lucerne	Luzerne
Pisum sativum Linn.	Pea	Erbse
Sinapis alba Linn.	Mustard	Weisser Senf
Solanum tuberosum Linn.	Potato	Kartoffel
Trifolium sp.	Clover	Klee
Triticum vulgare Host.	Wheat	Weizen

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Received for publication: February 27, 1958

Nematologica III (1958) : 283-294. E. J. Brill, Leiden

THE LIFE HISTORY OF THE CITRUS NEMATODE *TYLENCHULUS SEMIPENETRANS* COBB [1])

BY

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The citrus nematode was first discovered in California by J. R. HODGES, horticultural inspector, and was reported in some detail by THOMAS (1913). That same year COBB (1913) studied this nematode and placed it in a new genus and named this species *Tylenchulus semipenetrans* on the basis of position in the root. The following year COBB (1914) published a description of the development and structure of the nematode. He described the following life stages: mature females and males, first-stage larvae, and second- or third-stage females. Measurements of the eggs were first reported by CARVALHO in 1942. He also indicated that the measurements of males, females, and larvae by COBB (1913) were too large. The molt in the egg, two types of larvae, and pre-parasitic females were described by GUTIERREZ in 1947 [2]).

The citrus nematode is known to occur in all the citrus-growing areas of the world. In a survey of California citrus groves, 92% of the groves examined were found to be infested (SPEARS 1956). BAINES *et al* (1952, 1954, 1957) reported that in California the growth of young lemon and orange trees was reduced from 10 to 60% and that yields of mature trees were reduced from 10 to 20% as a result of citrus nematode infestation.

Because of the economic importance of this pest and the fact that little is known about its development, a thorough study of the life history of the citrus nematode was undertaken.

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1) Paper No. 1031, University of California Citrus Experiment Station, Riverside, California.

2) Dr SCHUURMANS STEKHOVEN has kindly called to my attention a description of *Tylenchulus semipenetrans* in a Russian publication which I have not reviewed: KIRJANOVA, E. S. (1955) Kruglie tscherwi (Nematodi) parasiti rastenii — Akademia Nauk SSSR Leningrad. pp. 75-80.

MATERIALS AND METHODS

The determination of a molt in the egg was made by placing several individual eggs in a drop of water on a slide and covering with a coverslip. Gentle pressure applied to the coverslip with a needle ruptured many of the eggs without damaging the larvae. Eggs killed and fixed on the roots with acid fuchsin-lactophenol were also studied.

Hatching studies were carried out by removing egg masses from infected roots and placing them singly in depression slides filled with fresh tap water. The egg masses were teased apart and all the living nematodes were removed. The slides were incubated in moisture chambers at 75° F. The water covering the eggs was changed every 3 days by pipetting water from the slide with a fine-pointed pipette. The slides were examined under a stereoscopic microscope at 24-hour intervals, and newly hatched larvae were removed to other depression slides filled with tap water and observed every 24 hours for a period of 16 days. The water was changed as described above, and under these conditions little mortality of eggs or larvae occurred. This method proved especially satisfactory for studying the development of the male.

The females were studied by infecting small citrus seedlings in sterilized soil with a suspension of citrus nematode larvae freed of fungi by suspending in a 1 : 1000 $CuSO_4$ solution for 30 minutes and then centrifuging and rinsing twice in sterile tap water. Individual pots containing two seedlings each were harvested at weekly intervals. The seedlings were gently removed from the soil and killed and fixed in hot acid fuchsin-lactophenol. The various stages of the nematode on the roots were then determined under a stereoscopic microscope.

Large numbers of female larvae and young females were readily obtained by placing infested roots in Petri dishes and covering with tap water for 24 hours. They were also obtained from soil washings; but finely divided mica common to California soils hindered observations under the microscope. Most of the inactive fourth-stage larvae and young females failed to pass through any modification of the Baermann funnel technique, which removed the soil particles.

Permanent slides of the various stages were prepared from specimens fixed in 5% formalin and mounted in aniline blue-lactophenol. This was done so that all nematodes for measurement were mounted in the same medium. All measurements and drawings were made with the aid of a camera lucida, using an oil-immerson lens which gave a magnification of 2000 on the drawings. Determinations of alpha, beta, and

TABLE I

Average ratios (de Man formula) and excretory pore in percentage of body length of female and male citrus nematodes [1])

Stage	Alpha	Ratio Beta	Gamma	Excretory pore (% of body length)
Male: — second (0-24 hours)	21.7	3.78	5.75	53.4
	(19.4-24.5) [1]	(3.50-4.00)	(4.90-6.68)	(50.7-56.4)
third	22.6	3.76	8.39	57.8
	(19.2-25.2)	(3.41-4.00)	(7.25-9.49)	(54.2-59.4)
fourth	23.8	3.91	9.24	58.3
	(20.2-29.5)	(3.40-4.32)	(7.55-11.7)	(57.0-60.1)
mature	33.9	3.57	8.28	56.5
	(29.0-39.0)	(3.28-4.23)	(7.89-10.1)	(53.1-58.4)
Female: — second (0-24 hours)	26.9	3.37		53.9
	(24.5-31.0)	(3.0-3.60)		(51.8-55.9)
third	22.4	3.30		62.0
	(19.7-25.7)	(2.93-3.75)		(59.0-64.2)
fourth	20.6	3.03		69.0
	(18.3-23.5)	(2.52-3.49)		(65.7-74.8)
young	17.5	2.52		80.2
	(15.0-20.0)	(2.15-3.00)		(76.9-84.2)
mature, swollen	4.5	2.97		82.5
	(3.75-5.07) [2]	(2.80-3.12) [2]		(79.4-85.0) [2]

[1] Numbers in parenthesis are the minimum and maximum variation of 25 specimens, unless otherwise stated.

[2] Measurements of 10 females only.

gamma ratios of the de Man formula are presented along with data on the excretory pore in Table I. Since little overlap of the extremes occurred in the important differential stages, the data were not submitted to statistical analysis. All measurements given in the text are from 25 nematodes unless otherwise indicated.

Populations of the citrus nematode from roots of orange (*Citrus sinensis*), lemon (*Citrus limon*), lilac (*Syringa vulgaris*) and persimmon (*Diospyros lotus*) at Riverside were studied. In addition, populations from orange and lemon roots from Ventura County were compared with those from Riverside County.

RESULTS

Embryonic development. The first molt was observed in the egg. The development of the larva in the egg prior to the first molt was not studied. Although not distinct, the molt in the egg was detected in uncrushed stained eggs. The first-molt cuticle was evident soon after

the nematode reached full elongation. There were no remnants of a stylet in the first-molt cuticle; however, the second-stage larva developed a very prominent stylet while yet within the egg. The female and male larvae could be differentiated under oil immersion just prior to hatching from the egg, and under a low-power stereoscopic microscope immediately after hatching.

Hatching studies. The eggs of the citrus nematode are rather thin-walled and measure 67 × 33 μ. The adult female normally contains one or sometimes two eggs at one time. Most of the eggs observed in the female contained only one blastomere. If two eggs are present, one might have two or three blastomeres. A total of 1100 larvae were hatched in depression slides filled with water. The eggs came from 16 different egg masses and were produced by 75 females. All the eggs of each egg mass hatched within 12 to 14 days. In several instances individual eggs retained within the body of the recently-killed females developed and hatched within this time. If the last eggs to hatch were the last eggs laid, then the length of time for maturation of the eggs in water was approximately 14 days at 75° F.

Two distinct forms of larvae hatched from the egg masses, one shorter in length and larger in diameter than the other. The former always developed into mature males within one week. The latter failed to change until after a sufficient feeding period on a suitable host. Twenty-six per cent of all the larvae hatched developed into mature males.

Second-stage male larvae. The second-stage male larvae are easily overlooked when ordinary soil and root sampling techniques are employed. The larvae pass through this stage within 48 to 72 hours after hatching and usually complete this stage before emerging from the egg mass.

The second-stage male larvae average 307 μ (284-344 μ) in length. The esophagus is shorter and the diameter of the body is greater than in the second-stage female larvae (Fig. 1, A and B). These differences are considered significant, since the extreme variations meet and do not overlap. The excretory pore (Table I) is located slightly posterior to the middle of the body and remains nearly constant during the rest of the life stages. The stylet is prominent with conspicuous basal knobs and averages 12 μ (11-13 μ) in length.

The character of the tail is best described by the diagrammatic drawings in Fig. 1. The two clear areas in the posterior region are manifestations of the genital primordium located posterior to the excretory pore, and the tissue around the anus. In comparison, the female has

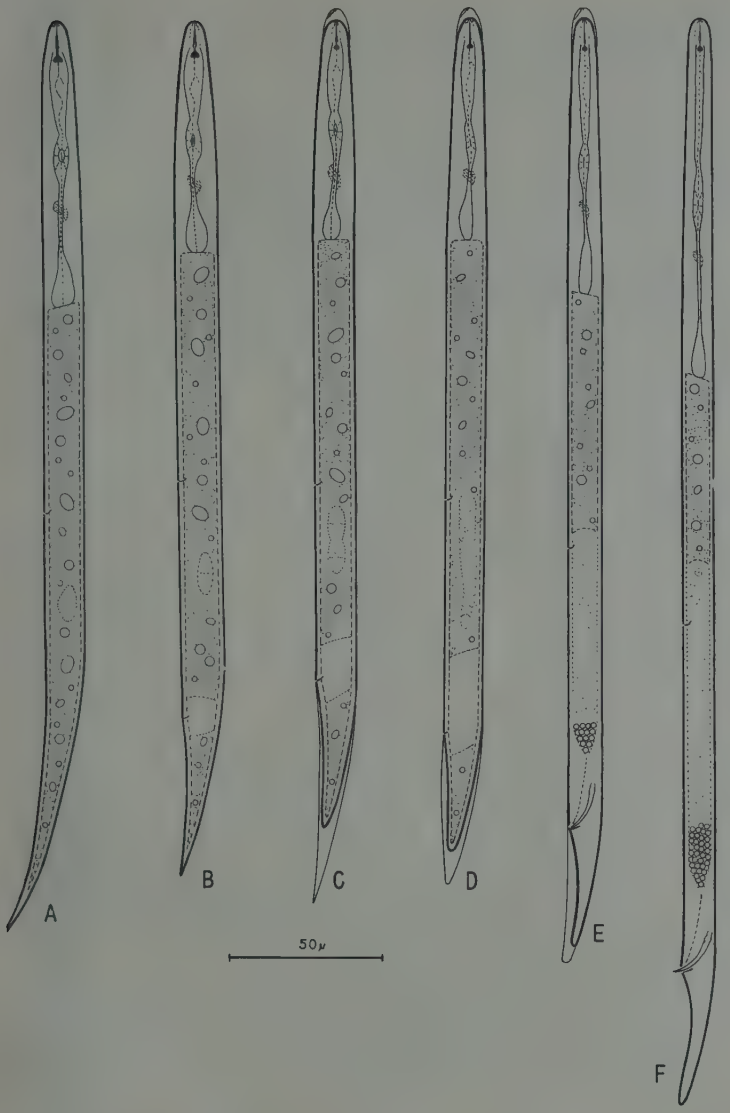

Fig. 1. *Tylenchulus semipenetrans* Cobb. A. Second-stage female larva. B. Second-stage male larva. C. Third-stage male larva. D. Fourth-stage male larva. E. Young male within fourth-molt cuticle. F. Mature male.

only one clear area near the middle of the body. The genital primordium, which develops very rapidly, has from two to four cells, the number depending upon its age.

The first evidence of molting consists of a gradual cessation of the body movements and a dissolution of the basal portions of the stylet, usually 24-28 hours after hatching. The male nematode shows only sluggish movements during the entire molting period.

Third-stage male larvae. The third-stage male larvae are approximately 281 μ (250-309 μ) long — somewhat shorter than the second-stage larvae. The esophagus is shorter and the width of the body is narrower, as reflected in the ratios shown in Table I. The tail is much shorter in this stage than in the second stage. The genital primordium is four- to eight-celled. The clear areas in the posterior portion of the body are larger (Fig. 1, C).

The stylet is inconspicuous with poorly developed basal knobs averaging 11 μ (10-12 μ) in length. The anterior portion of the second-stage stylet is molted with the cuticle. The third molt is complete 108-144 hours after hatching.

This is probably the stage of the male that Cobb (1914) describes as a first- or second-stage male larva. The developing spicule which he describes was not seen except in the later stages. In some specimens a definite canal can be seen forming through the tissue of the anus.

Fourth-stage male larvae. The fourth-stage male larvae average 290 μ (263-325 μ) in length. In this stage there are many indications of approaching maturity (Fig. 1, D). The genital primordium is greatly enlarged, stretching from the excretory pore nearly to the developing spicule. The testis consists of numerous cells, but no mature sperm were observed. An indistinct spicule can be seen developing, and at this stage the entire posterior region of the body is nearly clear. There is not much change in esophagus length and body width, but the esophagus shows signs of becoming degenerate. The stylet is inconspicuous, averaging 11 μ (10-12 μ) in length.

Mature males. After molting, the male undergoes large changes in length and width, becoming long and slender. The first mature males were found 180 hours after hatching. Those observed in depression slides retained all three molt cuticles for 16 days. Males collected from root and soil samples do not retain their molt skins. Activity in the soil probably aids in removing molt cuticles. The mature males averaged 370 μ (330-411 μ) in length, much longer than in any previous stage (Fig. 1, E and F). This increase in length must be uniform throughout

the length of the body since the position of the excretory pore remains unchanged. The esophagus is shriveled and nonfunctional. The stylet is inconspicuous and much reduced in size, averaging 11 μ (10-12 μ) in length. The testis is well developed and has abundant mature sperm. It occupies approximately 36% of the total body length.

Second-stage female larvae. The life stages of the female are less easily observed than those of the male because the second-stage female larvae fail to undergo molting in the absence of a feeding period on a suitable host. In greenhouse inoculations it required approximately 14 days for the second-stage female to become associated with the root, begin feeding on the epidermal cells, increase in diameter, and begin the second molt.

Cobb (1914) stated that the gregarious habit of this nematode was due to the proximity of a food supply for the newly hatched larvae. This may be true where there is reinfestation within the colony, but in new root infestations it is more likely to be due to the congregation of second-stage larvae under small pieces of soil, organic debris, and sluffed cortical tissue still clinging to the roots. Frequently 25-50 larvae were found under these small protective covers within one week after inoculation. Very few single nematodes are associated with the unprotected roots. These covers may serve as a protection against environmental changes and for mechanical supports needed in feeding and penetrating the roots.

Newly hatched larvae are dark throughout the intestinal region, later becoming characterized by dark and light banding of the intestinal area. The area of light banding increases when the larvae go without feeding. How long the nematode can go without feeding and still complete its life cycle is not known. Active citrus nematode larvae were recovered by Baines (1950) from cans of soil held at 59° F for 2½ years, but only after 2½ months at 91° F. It appears that this is the most persistent stage of the citrus nematode.

The second-stage female larvae average 323 μ (295-364 μ) in length. The stylet is approximately 13 μ (12-14 μ) in length. The esophagus is longer than in the second-stage male larvae, and the body diameter is less than in the male (Fig. 1, A and B). The genital primordium consists of two cells and is located just posterior to the excretory pore. There is no functional anus.

Third-stage female larvae. The third-stage female larvae average 342 μ (312-375 μ) in length, slightly longer than the second-stage larvae (Fig. 2, A). The greatest change, as mentioned earlier, is the increase

in body diameter indicated by the much smaller alpha ratio (Table I). The esophagus is longer, larger, and better developed than in the second stage, suggesting that the nematode feeds during this stage. A well-developed, robust stylet is present, with an average length of 13 μ (12-14 μ).

The excretory pore is located at 59.0-64.2% of the body length (Table I). The genital primordium is four-celled and enlarged, usually opposite the excretory pore. The beginning of the vulva was detected by using aniline blue stain. The tail is more bluntly rounded than in the second stage.

Fourth-stage female larvae. The fourth-stage larvae average 326 μ (291-364 μ) in length — about the same length as the second-stage female larvae (Fig. 2, B). The esophagus is a little longer and more fully developed than in the third-stage larva. This stage has a robust stylet averaging 13 μ (12-14 μ) in length. The genital primordial cells are still four in number, but greatly enlarged, extending anterior to the excretory pore.

The excretory pore is located at 65.7-74.8% of the body length and has increased greatly in size. There is a definite swelling at the opening, and in some specimens the duct can be traced to an inner sinus which Cobb (1914) called a renette cell.

The fourth-stage females and young females were found on roots 21 days after inoculation. A few fourth-stage larvae were found with their heads three to four cells deep in the root. However, most of the fourth-stage and young females were found on the surface of the root. There were no indications that they were actively penetrating the root. The female completes its molting approximately 7 days after the beginning of the second molt.

Young females. Young females have a well-developed vulva and an immature ovary. The young females average 290 μ (250-360 μ) in length — the shortest female stage. The stylet averages 13.5 μ (12-15 μ) in length. There is a slight swelling at the vulva and a dorsal-ventral curvature of the posterior portion of the body (Fig. 2, C and D). This stage is described by Cobb (1914) as having numerous cells in the ovary, but in all the specimens observed, only four enlarged cells were found. The excretory pore is at 76.9-84.2% of the body length and remains so for the rest of its development. The vulva is well developed.

Larvae at this stage remain on the surface of the roots for a very short time. Within a week the females had penetrated to the pericycle

Fig. 2. *Tylenchulus semipenetrans* Cobb. A. Third-stage female larva. B. Fourth-stage female larva. C. Young female within fourth-molt cuticle. D. Young female.

of the root and were three-fourths to fully swollen. Eggs were present five weeks after inoculation at 25° C. Measurements of 10 egg-laying females showed an average length of 375 μ (349-406 μ), considerably

shorter than that reported by Cobb (1913). The morphology of mature females has been described by Cobb (1913, 1914) and Gutierrez (1947); little more needs to be said about them. The complete life cycle from egg to egg required six to eight weeks. This is in agreement with Cobb (1914), Carvalho (1942), and Gutierrez (1947).

Hand-picked second-stage female larvae developed and reproduced on citrus roots in the absence of males. Both male and female larvae were produced by the unfertilized females. Preliminary tests suggest that fertilization or lack of fertilization may influence sex ratios.

DISCUSSION

This appears to be the first report of separation of the male and female of any plant-parasitic nematode in the second larval stage. Previously, Raski (1950) reported sexual differentiation of *Heterodera schachtii* Schmidt in the third larval stage. This prompted the question of whether or not males were essential for reproduction, and if so could the genetics of this organism be studied? It was easy to determine that males were not necessary for reproduction by separating males and females in the second larval stage and infesting citrus seedlings with second-stage females. The role of the male in reproduction is under further study; likewise, the effects of environmental factors. In 1947, Gutierrez concluded that external factors had a powerful influence in the determination of sex in populations of the citrus nematode.

Many workers have overlooked the fine work of Gutierrez (1947). He described the first molt within the egg and the young female as the pre-parasitic stage. He also observed two distinct types of citrus nematode larvae which he thought might be male and female. His description, however, indicated that he was comparing third-stage males with second-stage females. No measurements or details of life stages were given.

The measurements obtained in this study of the various stages of the citrus nematode are much shorter than those reported by Cobb (1913). He did not say how many nematodes he measured, only that populations from all over the world were examined. Carvalho (1942) reported measurements of the citrus nematodes in Argentina similar to those of Cobb (1913) but shorter. Specimens of the size reported by Cobb (1913) were found in this study but were the extreme variation rather than the mean.

No anus was observed even in the youngest of second-stage female

larvae. A deteriorated anus was described in the young female by Cobb (1914), but Gutierrez (1947) concluded that there was no anus present in the female. Although the female has no functional anus, it does have a highly developed excretory system. An anal opening was observed in the male larva and it became the genital opening.

The stages of the life history and the observed differential characteristics of the citrus nematode were constant in the populations studied. It seems likely that this will also hold true for populations of the citrus nematode in other parts of the world; if so, they may be useful in future investigations.

The life cycle from egg to egg is influenced by temperature and "available" moisture. The temperatures used for these studies were the lowest optimum temperatures reported by Baines (1950). He found the optimum temperature for infection and reproduction of the nematode in constant-temperature tanks to be between 77° and 88° F. The time of year, then, will have an influence on the length of time required to complete the life cycle. The interval of time between irrigations also has an important bearing on the life cycle, since free water is necessary to flush the larvae from the egg mass into the soil in search of new roots.

The author is very grateful for the help and cooperation of Dr S. A. Sher and Dr O. Paul Straubinger.

ZUSAMMENFASSUNG

Der Lebenszyklus des Citrusnematoden umfasst fünf Stadien und vier Häutungen. Die erste Häutung vollzieht sich im Ei. Die Larven im zweiten Stadium zeigten geschlechtlichen Dimorphismus. Die männlichen Larven begannen ihre Häutung 48 Stunden nach dem Ausschlüpfen und vollendeten ihre Häutung innerhalb von 180 Stunden. Weibliche Larven benötigten vor Beginn ihrer Häutung eine sieben- bis vierzehntägige Fütterungsperiode auf einem geeigneten Wirt. Ihre Häutung war nach 21 Tagen beendet. Die weiblichen Larven blieben an der Wurzeloberfläche und drangen erst als junge Weibchen ein. Die Männchen waren für die Reproduktion unnötig. Der Lebenszyklus von Ei zu Ei dauerte sechs bis acht Wochen.

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Received for publication: March 19, 1958

Nematologica III (1958) : 295-300. E. J. Brill, Leiden

DIPLOGASTER LEPIDUS N. SP. UND DER SCHLÜSSEL DER *DIPLOGASTER*-ARTEN VON UNPAARIGEM OVAR

VON

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Die im folgenden besprochene neue *Diplogaster*-Art wurde in Schleimüberzügen einiger frischen Weinreben aus der Staatswirtschaft von Balatonboglár (Ungarn) gefunden. Auf dem Schnittoberflächen dieser Reben befanden sich reiche Bakterienkulturen, in denen eine ungeheure Menge von Nematoden zu beobachten waren. Hieraus kam *Diplogaster lepidus* in grosser Anzahl hervor, und zwar in der Gesellschaft von *Rhabditis oxycerca* de Man, 1895 und *Diplogaster paulyi* Fuchs, 1933.

Diplogaster (Eudiplogaster) lepidus n. sp.

♀: L = 0,307-0,497 mm; a = 22,4-25,0; b = 4,1-4,7; c = 3,8-4,3; V = 51,1-53,5 %

♂: L = 0,357-0,408 mm; a = 23,5-27,4; b = 3,7-3,9; c = 4,7-5,0.

Holotypus ♀: L = 0,461 mm; a = 23,2; b = 4,4; c = 4,1; V = 53,0 %.

Allotypus ♂: L = 0,360 mm; a = 23,7; b = 3,9; c = 5,0.

Diplogaster lepidus ist eine sehr kleine Art mit haarfein ausgezogenem Schwanz. Die Kutikula ist ausserordentlich dünn (0,5-0,8 μ breit) und sehr fein quergeringelt; die Ringelbreite beträgt bei der Mitte des Körpers 0,9 μ. Der breit abgerundete, nicht abgesetzte Kopf besitzt 6 sehr kleine Borstenpapillen und beim ♂ noch 4, etwas hinter dem Mundhöhlenbeginn liegende feine Borsten. Die Mundhöhle ist klein, etwa so lang wie breit, zweiteilig. Der vordere Mundhöhlenabschnitt besteht aus dem Cheilo- und Protorhabdion, den hinteren Teil bildet das Telorhabdion. Zwischen dem ersten, weiteren Teil und dem hinteren, schmalen, zylindrischen Abschnitt befindet sich das gezahnte Metarhabdion. Das Cheilorhabdion ist ausserordentlich fein längsgerippt und es übertrifft nach hinten das Vorderende des Protorhabdions. Beide sind stark chitinisiert und erweitern sich nach hinten bemerkbar. Das

Metarhabdion trägt einen gut ausgebildeten, stark chitinisierten, krallenartig nach vorn gebogenen Dorsalzahn und einen viel schwächeren, oft undeutlichen, kaum chitinisierten, gerade zugespitzten Subventral-

Abb. 1. *Diplogaster lepidus* n. sp. A: Kopfregion, 1600 ×; B: Vorderkörper, 750 ×; C: Vulvagegend, 750 ×; D: Schwanz des ♀, 750 ×; E: Analgegend des ♂, 1600 ×.

zahn. Beide Zähne liegen in derselben Höhe und sind beweglich bzw. ausstülpbar. Die linke, subventrale Platte des Metarhabdions ist wegen der Kleinheit des Tieres undeutlich. Der am wenigsten chitinisierte Mundhöhlenteil ist das Telorhabdion, das zwei kleine, subventrale End-

knöpfe besitzt. Die grösste Breite der Mundhöhle beträgt bloss 2,3-2,5 μ.

Das Seitenorgan ist queroval, nicht auffallend; es befindet sich hinter der Mundhöhle (beim ♂ ist es grösser als beim ♀). Der Vorderteil des Ösophagus ist stark muskulös und stets länger (59-61 % der Gesamtösophaguslänge) als der hintere Teil. Die Breite des kräftigen Mittelbulbus beträgt 12-13 μ (♀) bzw. 10 μ (♂); er hat einen wohl chitinisierten Valvularapparat. Kaum kleiner ist der Endbulbus, dessen Drüsenkerne wohl sichtbar sind. Der Exkretionskanal öffnet sich zwischen den beiden Bulbi. Der dünnwändige Darm besitzt ein weites Lumen und zahlreiche Blasen. Die Länge des Enddarmes ist etwa 1½mal so gross wie die anale Breite; er erweitert sich proximal.

Das weibliche Geschlechtsorgan ist unpaarig, prävulvar. Die Vulva befindet sich ein wenig hinter der Körpermitte, ihre Lippen ragen leicht hervor. Die Gonade ist 3,9-5,8 mal, das zurückgebogene Ovar 2,3-5,8 mal so lang wie der betreffende Körperdurchmesser. Ein verhältnismässig wohl entwickelter postvulvarer Uterussack von der Länge von 1,3-1,6 maliger Körperbreite ist vorhanden. Seine Grösse beträgt 1/5 bis 1/3 der Entfernung Vulva-Anus. Er dient als Receptaculum seminis und enthält zahlreiche rundliche Spermien. Der Uterus birgt jeweils nur ein sehr grosses Ei (62 × 15 μ), dessen Länge 3,0-3,2 mal grösser ist als die korrespondierende Körperbreite. Die Entfernung Vulva-Anus ist etwas kleiner als der Schwanz.

Der unpaarige Testis beugt sich kurz zurück. Die Spikula sind schlank, leicht gebogen, 21,0-22,2 μ lang (etwa 1,8 mal so gross wie die Analbreite). Sehr kennzeichnend ist für sie, dass ihre Distalende haarfein ausgezogen und kaudalwärts abgebogen ist. Das verhältnismässig grosse Gubernakulum hat eine Länge von 10,5-11,5 μ. Seine beiden Enden sind gleichfalls leicht kaudal gebogen. Die Zahl der Geschlechtspapillen beträgt 8 Paare: 2 Paare von ihnen liegen prä-, 1 Paar ad- und 5 Paare postanal. Die Präanalpapillen stehen subventral, die adanalen sublateral und 4 Paare der postanalen subventral, 1 Paar derselben aber subdorsal. Die vorletzten 2 Postanalpapillenpaare sind kleiner als die übrigen.

Der Schwanz ist bei den beiden Geschlechtern ähnlich, lang, fein auslaufend, beim ♂ verhältnismässig ein wenig kürzer als beim ♀; 8,7-9,6 mal (♀) bzw. 6,1-7,1 mal (♂) so lang wie die anale Körperbreite. Die deutlichen Phasmidien liegen im letzten Viertel des dickeren Schwanzabschnittes.

Kurze Diagnose: *Diplogaster* (*Eudiplogaster*) mit geringer

Körpergrösse, fein geringelter Kutikula, winzigen Lippenborsten, zweiteiliger Mundhöhle, krallenartig gebogenem Dorsalzahn, querovalem Seitenorgan, kräftigem Mittelbulbus, unpaariger Gonade, verhältnismässig wohl ausgebildetem hinterem Uterusast, sehr grossem Ei, charakteristischem Spikularapparat, 8 Paar Geschlechtspapillen, langem, fadenartig zugespitztem Schwanz und mit deutlichen Phasmidien.

Diese neue Art gehört der Paramonovschen Untergattung *Eudiplogaster* an und steht auf Grund des Mundhöhlenbaues und des unpaarigen weiblichen Geschlechtsorgans *Diplogaster systenocori* Körner, 1954 am nächsten. Sie unterscheidet sich aber davon durch die geringere Körpergrösse, den kräftigeren, krallenförmigen Dorsalzahn, den längeren Uterusblindsack, die weiter vorne stehende Vulva, den andersartigen Spikularapparat und den viel längeren, sehr verdünnten Schwanz. Ihre Abweichung von den anderen *Diplogaster*-Arten von unpaariger Gonade ist aus dem nachfolgenden Schlüssel ersichtlich.

Typischer Fundort: Balatonboglár in Ungarn, Bakterienüberzug von Weinreben (*Vitis vinifera* L.).

Typische Exemplare: 23 ♀ und 3 ♂ vom obigen Fundort, in der Sammlung des Verfassers.

Biologie: Die Art wurde in den erwähnten Baktierienüberzügen in grosser Menge angetroffen, obzwar die Leitart dieser Kulturen eine andere Nematoden-Art, *Rhabditis oxycerca* de Man war. Die Bewegung von *Diplogaster lepidus* ist ziemlich lebhaft, doch nie in so grossem Masse wie bei *Diplogaster paulyi* Fuchs. Die neue Art ist ovovivipar, indem die embryonale Entwicklung bereits innerhalb des im Uterus liegenden Eies beginnt. Obwohl sie zur selben Zeit nur ein Ei erzeugt, gedeiht sie ausgezeichnet. Sie ernährt sich höchstwahrscheinlich von Bakterien, da ich gröbere Nahrungsreste im Darmkanal nie sah und keine für sie geeigneten Nahrungsstoffe anderer Art in den Kulturen zu beobachten waren.

BESTIMMUNGSSCHLÜSSEL DER *DIPLOGASTER*-ARTEN VON UNPAARIGEM OVAR

1 Weiblicher Schwanz stark verjüngt, haarfein ausgezogen, mehr als 6mal so lang wie die anale Körperbreite (*c* kleiner als 6) 2
— Weiblicher Schwanz konisch, nicht haarfein ausgezogen, höchstens 5mal so lang wie die anale Körperbreite (*c* = 7 oder darüber) 9
2 Vulva vor 2/3 der Körperlänge (*V* kleiner als 60 %) 3
— Vulva in 2/3 der Körperlänge oder dahinter (*V* grösser als 60 %) . . . 7
3 Kleine Arten, Körper höchstens 0,6 mm lang 4
— Grössere Arten, Körper länger als 0,6 mm (bis über 1,5 mm) 6
4 Mundhöhle etwa so lang wie breit, beide Zähne in derselben Höhe; Telorhabdion röhrchenartig. — ♀ : L = 0,307-0,497 mm; a = 22,4-25,0; b =

4,1-4,7; c = 3,8-4,3; V = 51,1-53,5 %. — ♂ : L = 0,357-0,408 mm; a = 23,5-27,4; b = 3,7-3,9; c = 4,7-5,0 . . . . . *lepidus* n. sp.

— Mundhöhle schlank, bedeutend länger als breit. Dorsalzahn vor dem Subventralzahn; Telorhabdion klein, unbedeutend 5

5 Dorsalzahn kräftig, vom Grund der Mundhöhle bis zur Mitte derselben vorspringend; Schwanz 25-30 % der Körperlänge. — ♀ : L = 0,50-0,56 mm; a = 28-31; b = 5,0-5,9; c = 3,5-3,7; V = 49,0-54,6 %. — ♂ : unbekannt . . . . . *minor* Cobb, 1893

— Dorsalzahn klein, die halbe Länge der Mundhöhle nicht erreichend; Schwanz grösser als 30 % der Körperlänge. — ♀ : L = 0,44-0,56 mm; a = 27-29; b = 6,6-7,4; c = 2,7-2,9; V = 45,0-45,5 %. — ♂ : L = 0,6 mm; a = 38,5; b = 6,7; c = 3,1 . . . . . *parvus* Cobb, 1893

6 Mittelbulbus schlank, schwach entwickelt; Gubernakulum auffallend verdickt, stämmig. — ♀ : L = 1,00-1,67 mm; a = 24,4-40,7; b = 4,7-5,8; c = 2,0-4,1; V = 46,4-57,6%. — ♂ : L = 0,85-1,22 mm; a = 25,6-46,7; b = 4,7-7,1; c = 2,0-5,9 (Syn. *D. inaequidens* Paesler, 1946) [1] . . . . . *graciloides* Skwarra, 1921

— Mittelbulbus kräftig, rundlich; Gubernakulum schlank. — ♀ : L = 0,65-1,00 mm; a = 26-32; b = 5,5-7,0; c = 2,5-3,2; V = 52-57 %. — ♂ : L = 0,63-0,70 mm; a = 30; b = 6; c = 3. (Syn. *D. irregularis* Paesler, 1946) . . . . . *monhysteroides* Bütschli, 1874

7 Spikula ausserordentlich gross und schlank, 2-2½ Analbreiten lang; postvulvarer Uterussack fehlend. — ♀ : L = 0,8-0,9 mm; a = 18-22; b = 5-7; c = 4-5; V = 69,8-71,0 %. — ♂ : L = 0,7 mm; a = 34; b = 6; c = 6-7 . . . . . *gracilis* Bütschli, 1876

— Spikula nicht auffallend gross, höchstens 1½ Analbreiten lang; ein kurzer postvulvarer Uterussack vorhanden 8

8 Männchen mit 2 Paar Präanalpapillen; Spikula 1½ Analbreiten lang, grösser als der vordere, verdickte Schwanzteil. — ♀ : L = 0,61-0,68 mm; a = 22-27; b = 6-7; c = 5,2-5,4; V = 63,2-65,6%. — ♂ : L = 0,57-0,61 mm; a = 27-32; b = 7; c = 6-7 . . . . . *mikuschi* Fuchs, 1938

— Männchen mit 3 Paar Präanalpapillen; Spikula etwa so lang wie die Analbreite, kürzer als der vordere, dicke Schwanzteil. — ♀ : L = 0,53-0,65 mm; a = 31,7-36,0; b = 4,7-6,3; c = 2,1-2,6; V = 47,8-50,0 %. — ♂ : L = 0,56-0,59 mm; a = 32,0-35,8; b = 6,0-6,4; c = 2,4-2,5 . . . . . *stammeri* Weingärtner, 1955

9 Vulva vor 2/3 der Körperlänge; beide Zähne in derselben Höhe. — ♀ : L = 0,67-0,77 mm; a = 26,8-32,2; b = 4,8-5,2; c = 6,9-9,4; V = 59,2-60,9 %. — ♂ : L = 0,43-0,62 mm; a = 24,1-28,3; b = 3,5-4,5; c = 13,2-16,4 . . . . . *systenoceri* Körner, 1954

— Vulva in 3/4 der Körperlänge oder weiter hinten; Dorsalzahn vor dem Subventralzahn. — ♀ : L = 0,75-0,95 mm; a = 14-15; b = 6,5-6,7; c = 9,0-10,5; V = 75-80 % — ♂ : L = 0,61-0,80 mm; a = 14-15; b = 5,5-5,6; c = 8,5-9,5 . . . . . *secundus* Bovien, 1937

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1) *Diplogaster inaequidens* Paesler, 1946 ist mit *Diplogaster graciloides* Skwarra, 1921 identisch. Obwohl die Beschreibung von Skwarra ziemlich unvollständig ist, stimmen alle von ihr erwähnten Merkmale (lange, schmale Mundhöhle, schwach entwickelter Mittelbulbus, stark gebogene, stämmige, proximal geknöpfte Spikula und kurzes, auffallend verdicktes Gubernakulum), sowie die Körpermasse mit der Beschreibung von Paesler (1946), ja auch mit der von Weingärtner (1955) sehr gut überein. Auch die Abbildungen des Spikularapparates sind bei den erwähnten Verfassern einander entsprechend. Ich synonymisiere daher die Paeslersche Art mit Skwarras *graciloides*.

SUMMARY

A new species of *Diplogaster* is described from bacterial slime on the rut surfaces of vines. Diagnosis: Body small, cuticle very finely striated. Lips with minute papillae. Stoma consisting of two parts, cheilorhabdion longer than protorhabdion, dorsal tooth strong, well chitinised, hook-like, right subventral tooth weak, acute, indistinct, left subventral plate invisible. Amphids oval, transverse. Both bulbs large. Female gonad single, anterior; posterior branch of uterus relatively well developed, longer than body diameter; egg very large. Spicules and gubernaculum characteristic; 8 pairs of sexual papillae on the male tail. Tail long, filiform, phasmids distinct. A key to *Diplogaster* species with unpaired gonads is given.

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Eingegangen: 27. März 1958.

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SOME REMARKS ON THE STATUS OF THE SUBFAMILY DOLICHODORINAE, WITH DESCRIPTION OF *MACROTROPHURUS ARBUSTICOLA* N.G., N.SP. (NEMATODA: TYLENCHIDAE).

BY

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The systematic position of the two genera *Dolichodorus* Cobb, 1914 (species *D. heterocephalus* Cobb, 1914, *D. obtusus* Allen, 1957 and *D. similis* Golden, 1958) and *Belonolaimus* Steiner, 1949 (only species *B. gracilis* Steiner, 1949) has up to now not been definitely established. Chitwood & Chitwood (1950) united them into a new subfamily Dolichodorinae (family Criconematidae), while Thorne (1949) and Goodey (1951) placed *Dolichodorus* doubtfully in the Tylenchidae. *Belonolaimus* was not discussed by these authors.

The diagnosis of the subfamily by Chitwood & Chitwood reads: Cuticle coarsely striated, interrupted laterally by 3 or more lateral incisures; both sexes of moderate size, very elongate, eel-shaped; females with 2 ovaries, male with caudal alae terminal (*Dolichodorus*) or adanal (*Belonolaimus*) [1]).

Chitwood & Chitwood defined the family Criconematidae as having the stylet shaft elongated and the metacorpus greatly enlarged. Obviously for this reason they assigned Dolichodorinae to Criconematidae. However, both genera differ from the other members of this family, according to the original diagnosis by Thorne (1949), in several important characters. The ovaries are paired, the median bulb is rounded, not elongate, and off-set by constriction from the precorpus; the isthmus is well developed; the males possess a normally developed spear and oesophagus and large caudal alae; finally, the terminal oesophageal bulb of *Dolichodorus* is not reduced. All these characters point to the Tylenchidae and it is reasonable to place the two genera in that family. Not much stress should be laid on the elongate stylet: in both Cricone-

1) In the eight males studied by the writer the bursa enclosed the tail tip. Steiner's figure also shows a terminal bursa.

matidae and Tylenchidae spear length is very variable: Criconematidae from 15 μ (certain *Paratylenchus* species) to 150 μ (*Hemicycliophora gigas* Thorne, 1955); Tylenchidae from 4 μ (*Tylenchus infirmus* Andrassy, 1954) to 67 μ (*Tylenchorhynchus superbus* Allen, 1955). Even when stylet length is regarded in relation to oesophagus length, great differences remain. As to the enlarged corpus, this is clearly correlated with the long spear: when this is retracted, the oesophageal lumen gets coiled and the corpus should contain room for it. The same might perhaps hold true for the ampulla in the duct of the dorsal oesophageal gland near the orifice, which STEINER mentions as present in *Belonolaimus* and which, according to his figure, also seems to occur in *Dolichodorus*.

The subfamily diagnosis does not fit however. The cuticle of *Dolichodorus* is rather finely striated, and *Belonolaimus* has one lateral line. The only common character which separates them from the other Tylenchids is the elongate spear (the paired ovaries and the off-set lip region can hardly be considered taxonomic characters on the subfamily level). This character, however, is directly connected with mode of life and is of little taxonomic value. Thus the affinities between the two genera are superficial; the differences, on the other hand, are essential. The oesophagus of *Dolichodorus* has a terminal bulb, that of *Belonolaimus* a terminal lobe. This is a subfamily character in Tylenchidae, according to THORNE's system; *Belonolaimus* thus keys out to Hoplolaiminae, *Dolichodorus* to Tylenchinae.

Considering *Belonolaimus* first, this genus shows marked resemblances to the Hoplolaiminae through the shape of lip region and the strong cuticular annulation which extends around the tail tip. A link is *Hoplolaimus proporicus* J. B. Goodey, 1957, which has no lateral field (the annulation is interrupted only on the head and the tail end, thereby suggesting one lateral line) and only a few longitudinal ridges on the lip region. The chief differences between *Belonolaimus* and Hoplolaiminae are the tail length of the former genus, which is much greater than the anal body diameter, and the shape of the gubernaculum. In most of the specimens of *B. gracilis* studied, the terminal oesophageal lobe lies laterally, but after THORNE (1949) this occurs in Hoplolaiminae too.

ALLEN (1957) has emended the definition of *Dolichodorus*. The genus does not show any close affinities to other genera; in fact, the peculiar shapes of the bursa and the female tail place it somewhat apart. Even in *D. obtusus*, where the female tail is rounded, the larva possesses the same tail shape as the females of the other two species. It is

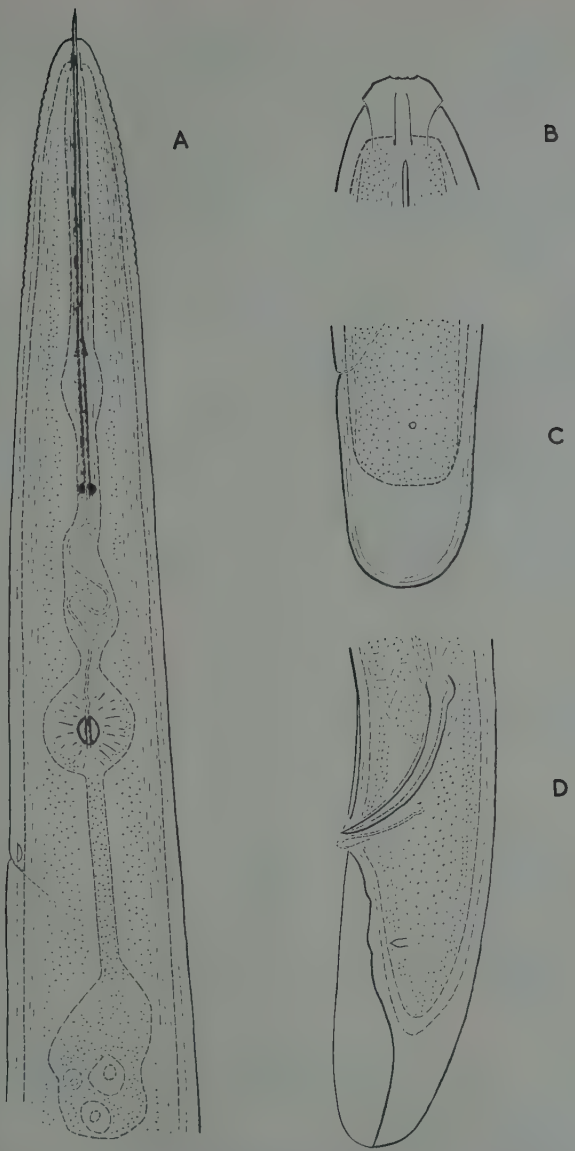

Fig. 1. *Macrotrophurus arbusticola* n.g.n.sp. A. Head and neck of female, × 750. B. Anterior end of female, medial view. with amphid apertures, × 1500. C. Female tail, lateral view, × 750. D. Male tail, sublateral view, × 750.

reasonably sure that *Dolichodorus* is not directly related to *Belonolaimus*. The subfamily Dolichodorinae should be abandoned: *Belonolaimus* should be assigned to Hoplolaiminae, *Dolichodorus* to Tylenchinae, as the differences mentioned are not sufficient to keep this genus in a separate subfamily.

The above considerations were occasioned by the finding of some nematodes with a very elongate stylet, representing a new genus of which a description will now be given and the systematic position briefly discussed.

MACROTROPHURUS N.G.

Definition: Tylenchidae. Nematodes of moderate size (1-2 mm); body in both sexes rather slender, attenuated in front, parallel behind. Lip region smooth, rounded, continuous with body contour. Stylet very long. Opening of dorsal oesophageal gland a short distance behind spear base. Middle bulb large, well set off from enlarged pre-corpus, with conspicuous valves. Isthmus rather long and narrow. Terminal bulb elongate. Tail rounded with very thick cuticle. Ovaries paired, opposed and outstretched. Vulva near middle of body. Male with well developed bursa.

M. arbusticola n. sp.

Type species:

Measurements: Females (n = 17): L = 1149 — 2053 μ; V = 44,7 — 50.0%;
a = 38.0 — 48,2; b = 6,3 — 10,2; c = 27,9 — 42,2; stylet 92 — 119 μ; G_1 = 21,6 — 31,5 %; G_2 = 21,7 — 27,3%.

Males (n = 8); L = 1044 — 1689 u; a = 39,5 — 57,5;
b = 6,2 — 8,5; c = 19,7 — 28,2; stylet 90 — 110 μ.

Female (holotype): L = 1634 μ; V = 48,8%; a = 48,2; b = 8,0; c = 42,2; stylet 99 μ; G_1 = 22,9 %; G_2 = 23,8 %.

Male (allotype): L = 1672 μ; a = 57,5; b = 8,5; c = 28,2; stylet 102 μ.

Cuticle rather thick, in 3 layers (best visible on female tail), the inner ones with fine striation. Striae on outer layer discernible only near head end. Lateral field indistinct, very broad (to 2/5 of body diameter), with 4 lines. An irregular row of pores along each of the inner lateral lines. Amphid apertures near base of lip region, easily visible in medial view. Lip sclerotization faint, only guiding apparatus of spear distinct.

Hemizonid immediately before excretory pore, 3,5 μ long. Excretory pore between the bulbs; in all specimens studied the duct was constricted as it passed through the cuticle. Metacorpus large, very muscular. Precorpus probably with ampulla in duct of dorsal gland. Terminal bulb with conspicuous gland nuclei.

Female: Vulva a transverse slit. Spermathecae broadly oval. Ovaries consist of one row of cells, except for a short zone near beginning of each. Rectum distinct. Phasmids somewhat clavate, anterior to middle of tail; in some specimens only slightly postanal. Caudal end of body inside cuticle almost truncate. Tail broadly rounded, sometimes slightly spatulate, with very thick cuticle (16 — 20 μ); only the innermost layer is thickened. One female contained an egg with dimensions 157 × 30 μ.

Male: Inner cuticular layer does not participate in formation of bursa. Cuticle on tail tip as thick as in female. Edge of bursa very indistinctly crenate. Bursa encloses tail tip. Spicules 29-35 μ, curved, with weakly set off cephalated proximal part. Gubernaculum hooked proximally, recurved distally.

Larva: Tail shape similar to that of adult female. Tail and oesophagus only slightly shorter than in adult. Measurements of one specimen: L = 630 μ; a = 28,9; b = 3,7; c = 15,3; stylet 75 μ.

Measurements based on two populations, one from Holland, the other from Switzerland. The Swiss specimens differed from the Dutch ones in some measurements as shown in the table.

	Dutch	Swiss
Females. n	11	6
L	1353-2053 μ	1149-1439 μ
b	7,7-10,2	6,3-7,0
c	31,2-42,2	27,9-35,8
Males. n	2	6
L	1672-1689 μ	1044-1437 μ
a	55,8-57,5	39,5-46,1
b	8,0-8,5	6,2-6,7
c	26,3-28,2	19,7-24,0

The differences in a, b and c are clearly of an allometric nature, as is shown by their correlation with body length (compare also the larval measurements given above). As, apart from size, no differences between the Dutch and Swiss populations could be detected, they are tentatively regarded as conspecific. The Swiss population might represent a geographical race, but it is equally possible that the smaller size

is caused by ecological conditions (cf. FRANKLIN 1957) and has no genetical base. Therefore at present no subspecific names are proposed.

Type specimens: Holotype: female in slide a 16; allotype: male in slide a 16; paratypes: nine females and one male in slides a 1, a 12, a 13 and a 14, in Plantenziektenkundige Dienst Collection at Wageningen, The Netherlands.

Type locality: Opheusden, The Netherlands.

Type habitat: Clay soil from orchard, around roots of pear and poplar.

Geographical distribution: The Netherlands; Switzerland, from tree nurseries near Zürich, among others from apple.

The specific name is derived from Latin arbustum = orchard and colere = to inhabit.

Systematic position. *Macrotrophurus* certainly shows some resemblances to *Dolichodorus* and *Belonolaimus*, and, if these two genera were so closely related as to be united into a separate subfamily, it would have to be included in this group as a third genus. It differs, however, strongly from both. The fine cuticular annulation and the terminal bulb separate it from *Belonolaimus*; the shape of female tail and male caudal alae from *Dolichodorus*; the continuous lip region from both. The structure of the oesophagus shows that it belongs to the subfamily Tylenchinae. From the genera of this group *Trophurus* and *Tylenchorhynchus* seem to be its nearest relatives. From the former genus *Macrotrophurus* differs by the elongate stylet and the paired ovaries. Now *Trophurus*, although monodelphic, has the vulva placed equatorially and therefore stands closely to didelphic forms. As moreover the peculiar shape of head and tail ends does not give the impression of being of strongly adaptive character, *Trophurus* may be regarded as the closest relative of *Macrotrophurus*. This view is expressed in the generic name.

Macrotrophurus differs from *Tylenchorhynchus* by the smooth lip region, the nearly smooth outer cuticle layer, the large amphid apertures and the very thick cuticle of the tail tip in both sexes.

The work was made possible by a grant from the L. E. B. Fund, Wageningen. The author also wishes to thank Dr. Ir. M. Oostenbrink for reading the manuscript and offering some valuable suggestions.

ZUSAMMENFASSUNG

Es wird eine neue Gattung *Macrotrophurus* beschrieben, charakterisiert durch stark verlängerten Mundstachel, paarige Ovarien, nicht abgesetzte Lippengegend

und abgerundeten weiblichen Schwanz mit sehr dicker Kutikula. Die Überprüfung der systematischen Stellung führte zur Schlussfolgerung, dass die Subfamilie Dolichodorinae aufgelöst werden sollte: *Belonolaimus* gehört zu den Hoplolaiminae, *Macrotrophurus* zu den Tylenchinae (in die Nähe von *Trophurus*). *Dolichodorus* ist am besten ebenfalls zu den Tylenchinae zu stellen, steht aber etwas isoliert.

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Received for publication: April 3, 1958

Nematologica III (1958) : 308-314. E. J. Brill, Leiden

OBSERVATIONS ON THE DEVELOPMENT OF *HETERODERA ROSTOCHIENSIS* WOLL. IN STERILE ROOT CULTURES

BY

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The use of a tissue culture technique in studying plant parasitic nematodes enables detailed observations to be made on a variety of problems. BERLINER & BUSCH (1914) attempted to culture *H. schachtii* on seedlings in agar and in the same year BYARS kept a root-knot nematode through one generation in aseptic culture. TYLER (1933) succeeded in carrying root-knot nematodes through several generations on tomato roots grown in nutrient agar and observed variations in the ratio of the sexes under different conditions. The susceptibility or immunity of host plants to nematode attack was investigated by LINFORD (1939). He was interested in the attractiveness of growing root and excised shoot tissue to a number of nematode species and found a more marked reaction in damp sand than in soil. His observation that the area behind the growing root tip was the most attractive was later confirmed by WIESER (1955, 1956). MOUNTAIN (1954) achieved successful infestations of sterile root tips of maize, tobacco and red clover grown in nutrient agar in Petri dishes with *Pratylenchus minyus*.

In a previous paper FENWICK (1956) dealt with the production of sterile viable larvae of the potato-root eelworm *H. rostochiensis*. The present paper is a record of observations on the penetration and development of sterile larvae in tomato roots grown under *"in vitro"* conditions.

TECHNIQUE

The methods of culturing the root tissues were those of WHITE (1943). Tomato seeds (variety Moneymaker) were sterilized by agitating for twenty minutes in a 5% bleaching powder solution and were

then washed twice in sterile water. They were sown singly in culture tubes containing approximately 15 ml of White's 0.75% agar medium per tube. After seven to ten days the radicle had grown sufficiently for the seedling to be removed, the root tip excised, and transferred to White's fluid medium, where growth was normal. Lateral root tips were removed weekly and transferred to fresh culture tubes of agar. The experiments described were made on excised tips from root cultures produced in this way, and kept at a temperature of 24° C.

Sterile eggs of *H. rostochiensis* were obtained by opening cysts and treating the free eggs with 20 volume hydrogen peroxide for from eight to sixteen hours, (FENWICK 1956). Although it is possible to hatch larvae from sterile eggs in sterile root diffusate, satisfactory results were obtained by introducing the sterile eggs directly to the root culture, where hatching was rapid. It did not matter whether the eggs were rubbed into the surface of the agar with a platinum loop or merely pipetted onto the surface; in the latter case the agar surface softened sufficiently for the eggs to sink down a few millimetres. The eggs were never introduced into the deeper layers of the agar, so that the later presence of larvae more than 2.5 cm below the surface must have been the result of hatching and active migration. Inoculation tests were made in this way in White's nutrient agar or in cultures of silver sand. When sand was used it was washed, flooded with the nutrient fluid and allowed to drain before being transferred to 6 × $^5/_8$ in. culture tubes for sterilizing.

Inoculation was accomplished by transferring a root tip, 1.0-1.5 cm long, to the surface of the sterile agar medium. When growth had commenced and the root tip had penetrated 2-3 cm, a suspension of sterile eggs was introduced to the surface of the medium. Successful inoculation could also be accomplished by adding the eggs to a young germinating seed. Hatching and subsequent migration always occurred whether or not the cotyledons and growing point had been removed.

In agar cultures it was possible to obtain rough estimates of hatch and migration by visual examination, but in sand cultures this was obviously impossible. Estimates of percentage hatch were obtained by shaking the contents of a tube with warm water to dissolve the agar, making up to a known volume and counting eggs and free larvae present in an aliquot.

The degree of penetration and development of larvae within roots was investigated by staining in acid fuchsin or cotton blue in lactophenol (GOODEY 1957).

RESULTS AND COMMENTS

Using these methods, large numbers of larvae usually hatched out of the sterile eggs a few days after inoculation. Counts of eggs and of free larvae in the agar showed that up to 70% of all larvae present had emerged in the presence of a growing root in about 4-7 days. Comparative figures for inoculations of tubes without roots were in the neighbourhood of 20-30% : it is therefore probable that root diffusate was being produced by the excised roots. Larval emergence in sand cultures was lower, due probably to variations in water content of the sand.

Four or five days after inoculation, concentrations of larvae in an active condition were usually visible at some of the root tips (Fig. 1). If very heavy concentrations of eggs were introduced into the tubes, the majority of the liberated larvae appeared to become inactive and die. As this apparent mortality was more pronounced at high densities, it may have been associated with local anaerobiosis. Fig. 2 shows a culture about seven days or more after inoculation. The concentration of larvae (many motionless) at root tip A is clearly visible and the path taken by them in reaching this point is marked by the trail of motionless larvae extending from the egg mass at the agar surface to the root tip. Such trails of larvae were frequently found, and an interesting feature is the apparent preference shown by larvae for certain root tips; thus, although the trail illustrated passes very close to the tip of root B, the larvae seem to have ignored it and to have concentrated at tip A. For no obvious reason some root tips appear to be less attractive than others to the hatched larvae. WHITE (1943) mentions that excised root tips are frequently encountered which are normal in appearance but which sink in the fluid medium and that these tips never grow well and are best discarded.

Minor concentrations of larvae were often found at isolated points along the main roots. Almost invariably, within a few days, lateral rootlets appeared at these points, indicating that larvae were anticipating the production of new endogenous rootlets (cf. WIESER 1955). In general larvae were attracted to lateral rootlets as well as to the main root tip which rapidly grew down to the bottom of the tube where oxygen concentration was presumably low; however after 14 days in culture some active larvae could still be found in these lower levels. With entire seedlings, the larvae congregated at the root tips, but with excised roots they concentrated a few millimetres behind the tip. This effect has also been observed by WIESER (1955) who found that larvae

of *Meloidogyne hapla* were repelled by excised tips 1-2 mms in length but attracted in increasing numbers as tip length increased to 8 mms; beyond this length attractiveness did not increase but remained at a high level.

Despite the apparently high mortality of larvae in agar, some remained viable long enough to penetrate the root tips. Penetration was frequently accompanied by localized swelling as seen in Fig. 3, although the effect is somewhat exaggerated in this photograph because the root has been crushed to expose the developing larvae within. Fig. 4 is a typical picture; the developing root tissue has hypertrophied to form a small gall containing the developing larvae. Although swellings sometimes occurred in the absence of invasions and conversely some invasions did not result in swelling, the association between the two was sufficiently good to serve as an indication of invasion when selecting roots for subculturing or staining. Galling also occurs under natural conditions when *H. rostochiensis* attacks tomato. Cyst production on this host is lower for any given initial level of infestation than on potato where no galling occurs (Jones 1957). *Meloidogyne* spp. cause more intense galling on tomato than does *H. rostochiensis* and are not themselves cyst formers. It is therefore probable that galling may be the result of specific interactions between parasite and host. The factors influencing it can at this stage be based only on conjecture, but should it be possible to isolate and culture galled tissues, then comparisons between their physiology and that of normal tissues might yield valuable information on physiological effects of parasitic nematodes on host plants.

Although developing larvae were found in all parts of the root system, the heaviest concentrations were in areas behind the root tips (O'Brien & Prentice 1931) or at the junctions of main and lateral roots. The area of root elongation behind the apical meristem is possibly more easily penetrable than other portions of the root system. Little is known about the mechanism of attractiveness. It is highly probable that attractive substances, which may or may not be identical with the hatching stimulus, are produced in this region, because it is around here that the greatest concentration of larvae is found in excised root tip cultures (c.f. Godfrey & Oliveira 1932, Gadd & Loos 1941). At root junctions larvae may be able to penetrate more easily as the lateral roots break through the cortex of the primary root.

The larvae which penetrated the roots usually did so within about 14 days from the time of maximum hatching, which was about 4-7 days

after inoculation. Moving larvae were sometimes found up to 40-42 days after inoculation but it is doubtful whether they were still capable of invading the root tissues.

The development of larvae inside the tissues appeared to proceed normally, and the time taken for the completion of each larval stage corresponded closely with that given by RASKI (1950) for the development of *H. schachtii* at the same temperature, and with CHITWOOD & BUHRER (1946) for *H. rostochiensis* under Long Island conditions. Some experiments were stopped at different times to estimate the level of larval emergence and also to investigate the degree of penetration and subsequent development. At the end of 14 days, all stages up to the third moult and including some early fourth stage larvae were found. Fig. 5 shows a second stage larva just before the moult, the pointed tail of the second stage being visible. A third stage larva is shown in Fig. 6. This photograph shows a feature which was frequently present, namely a brown patch in the cortex surrounding the head of the larva (c.f. O'BRIEN & PRENTICE 1931). This also occurred in non-sterile soil cultures of tomatoes (DONCASTER 1953). It would appear from these observations that the patches are in some way due to the nematodes and are not a secondary result of bacterial invasion. The head of the worm can be seen embedded in the root tissue.

Fourth stage larvae were usually visible about 14 or more days after inoculation and Fig. 7 shows a typical fourth stage larva photographed *in situ* through the wall of a culture tube. Fig. 8 shows a third stage larva (arrow) beside a young adult female photographed *in situ* approximately 24 days after inoculation, the shreds of the fourth stage cuticle are seen attached to the developing adult. Fig. 9 is a fully developed male worm still ensheathed within the third and fourth stage cuticles. The spicules are visible (arrow). This specimen was stained and mounted *in situ* in lactophenol. Fig. 10 shows a fully developed female approximately 42 days after inoculation. Shreds of the fourth stage cuticle can again be seen and the root is typically thickened just above the nematode.

Although the majority of larvae that succeeded in penetrating the roots developed normally into adult males or virgin females, some undeveloped second stage larvae were visible in the roots as late as 42 days after inoculation. These may have resulted from late invasion but it is more likely that they were early penetrators which had failed to develop due possibly to some abnormal conditions in the roots.

No free males were seen on the surface of the roots and it is assumed

Fig. 1. Tip of excised tomato root in sterile agar, with a concentration of newly hatched *H. rostochiensis* larvae.

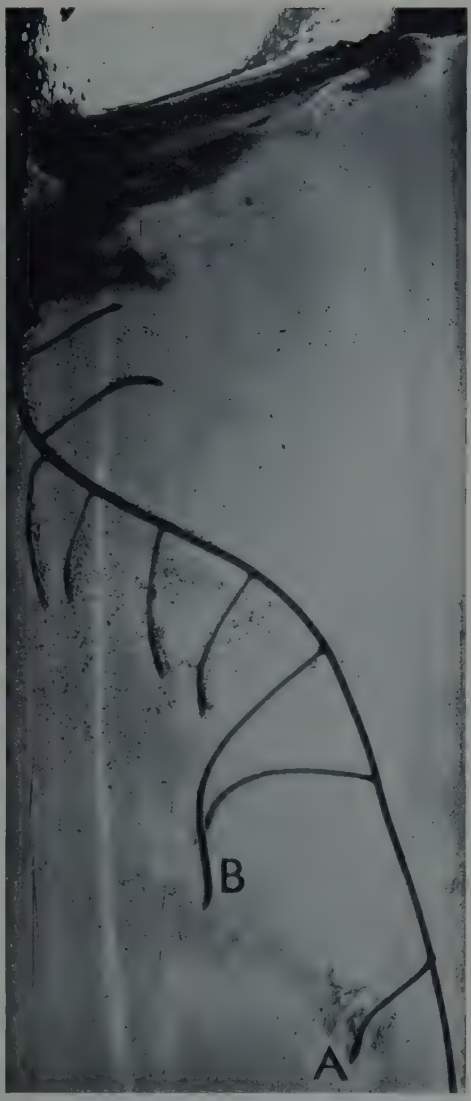

Fig. 2. Excised tomato root system in sterile agar culture. The distribution of nematode eggs and newly hatched larvae is shown.

Fig. 3. Tomato root crushed to show a concentration of infesting *H. rostochiensis* larvae and associated swelling behind the tip. (Stained preparation.)

Fig. 4. Infested tomato root showing gall (Photographed in sterile agar culture.)

Fig. 5. Crushed tomato root with infesting larvae, two of which are in the advanced second stage. (Stained preparation.)

Fig. 6. Tomato root showing cortical necrosis associated with a third stage *H. rostochiensis* larva.

Fig. 7. Tomato root with local swelling associated with a fourth stage *H. rostochiensis* larva. (Photographed in sterile agar culture.)

Fig. 8. Adult female and a third stage larva of *H. rostochiensis*. (Photographed in sterile agar culture.)

E. WIDDOWSON *et al*: *Sterile root cultures of Heterodera rostochiensis.*

Fig. 9. Adult male *H. rostochiensis in situ* in tomato root. (Stained preparation.)

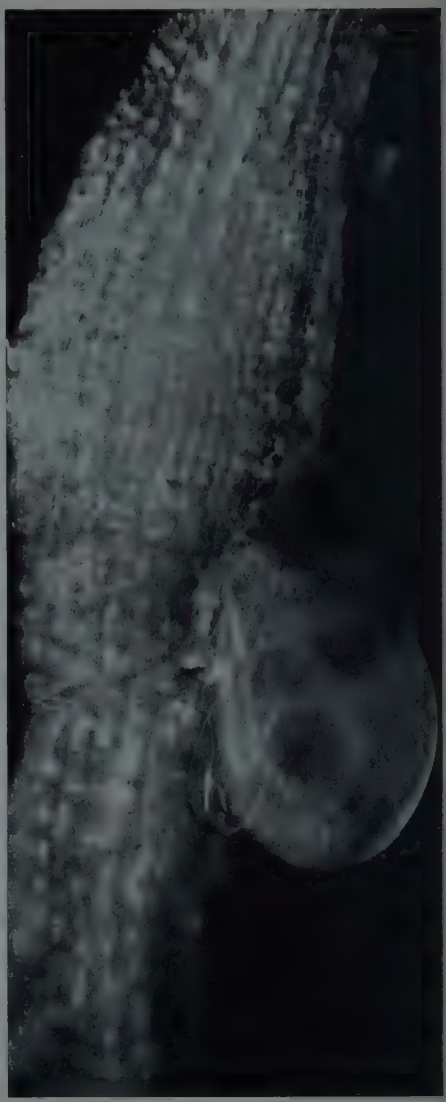

Fig. 10. Mature female *H. rostochiensis* in sterile agar culture, 42 days after inoculation

that on emergence they moved into the agar. A number of adult females were opened but contained no eggs. This may be due to conditions being unsuitable for fertilization or to inability of the host to maintain an infestation for so long a period under the experimental conditions. Raski (1950) found fully developed cysts of *H. schachtii* 21 days after penetration. Here, none was in a suitable position for direct microscopic examination before about 35 days after inoculation.

ZUSAMMENFASSUNG

Einzelheiten über die Technik des Ansetzens von Infektionsreihen mit sterilisierten Larven des Kartoffelnematoden in Wurzelgewebe werden mitgeteilt. Die Wurzeln wachsen in 0,75% Nähragar und Nematodeneier, deren Oberfläche mit 20 Vol.-% H_2O_2 sterilisiert sind, werden hinzugesetzt. Vier oder fünf Tage nach der Infektion wurden Gruppen aktiver Larven rund um die Wurzelspitzen gesehen. Der stärkste Befall tritt gewöhnlich innerhalb von 21 Tagen nach der Infektion auf.

Die Parasiten scheinen sich innerhalb des Gewebes zu erwachsenen Männchen und jungen Weibchen normal zu entwickeln. Keine freien Männchen wurden gefunden und keines der erwachsenen Weibchen, die geöffnet wurden, enthielt Eier.

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Received for publication: April 19, 1958

ERRATUM

Nematologica III, 3 (H. GOFFART & A. HEILING).

Seite 218, 10. Zeile von unten muss es „kein" (anstatt „klein") heissen.

In Tab. VI ist die Überschrift der Spalten folgendermassen abzuändern:

	Unbehandelt absolut	Behandelt	Diff.
Welke-Transp., mg/dm² in 20 Min.	109,7	88,2	-19,6%

usw.

Auf S. 225, 6. Zeile von unten muss es 80 ml/qm (anstatt 89 ml/qm) heissen.

Nematologica III (1958): 315-326. E. J. Brill, Leiden

UNTERSUCHUNGEN ÜBER DIE EIGENSCHAFTEN DER AUF *HETERODERA SCHACHTII* SCHMIDT AKTIVIEREND WIRKENDEN WURZELEXSUDATE VON *BRASSICA RAPA OLEIFERA* D.C.

VON

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Auf die Bedeutung der Wurzelausscheidungen verschiedener Pflanzen für die Aktivierung der enzystierten Larven des Rübennematoden (*Heterodera schachtii* Schm.) ist in zahlreichen Arbeiten hingewiesen worden, so zuerst von BAUNACKE (1922) und u.a. neuerdings von HIJNER (1951), GOFFART (1954) und WINSLOW (1954). Unsere Vorstellungen über das wirksame Prinzip in den Wurzelausscheidungen von Wirtspflanzen, durch die inaktive Larven dieser Nematodenart zum Verlassen ihrer Zysten angeregt werden, stützen sich jedoch bisher nur auf Vermutungen ohne experimentelle Bestätigung (RENSCH, 1925; WOOD u. SERRO, 1954; SWINK u. FINKNER, 1956, WALLACE, 1957). Wir versuchten daher die Wurzelexsudate [1]) von Rübsen (*Brassica rapa oleifera* D.C.), als einer häufig angebauten Wirtspflanze des Rübennematoden, aufzutrennen und die einzelnen Fraktionen durch ihre physikalisch-chemischen Eigenschaften und ihre Reizwirksamkeit zu kennzeichnen [2]).

BIOLOGISCHER TEST

Die für die Versuche verwendeten Nematoden entstammten einer Population von einem Feld bei Einbeck, Hannover, die an Zuckerrüben im Gewächshaus in den Jahren 1954-56 weiter vermehrt wurden. Durch kontrollierte Vermehrungszucht war es möglich, bei allen Schlüpfver-

1) Die Frage, ob es sich bei den von uns gewonnenen Stoffen im streng pflanzenphysiologischen Sinn um „Sekrete" oder „Exkrete" handelt, kann nicht eindeutig beantwortet werden (vgl. FREY-WYSSLING: Die Stoffausscheidung der höheren Pflanzen. Berlin 1935). Es soll daher im Folgenden nur allgemein von Wurzelexsudaten oder Wurzelausscheidungen gesprochen werden, worunter wir die Gesamtheit der von der Wurzel abgegebenen Stoffe verstehen.

2) Die hier berichteten Versuche wurden 1956 abgeschlossen (vgl. Chr. WINNER, Dissertation Göttingen 1956). Die Untersuchungen werden weitergeführt.

suchen volle, unbeschädigte Zysten in etwa gleichem Entwicklungsstadium zu verwenden und damit die Anzahl der Zysten je Versuchsglied auf 3 × 20 zu beschränken. In der Technik des Schlüpfversuches lehnten wir uns an die im Prinzip von BAUNACKE (1922) entwickelte und später von RADEMACHER (1930) verbesserte Methode an (Einwirkung der Testlösung auf die Zysten für zwei Tage; Auszählung der geschlüpften Larven in zweitägigen Abständen). — Nur eine Verdoppelung der Schlüpfrate gegenüber der unbehandelten Kontrolle in destilliertem Wasser wurde als positive Reaktion gewertet; Reize, die einen geringeren Schlüpfeffekt auslösten, wurden nicht als wirksam angesehen (Dementsprechende Flächenpunktierung bei den unten angeführten Wirkungsdiagrammen).

Für die Vergleichbarkeit der verschiedenen Versuchsserien war es notwendig, die relative Schlüpfbereitschaft jeder Zystenreihe zu ermitteln. Das geschah durch Zugabe einer aktivierend wirkenden Vergleichslösung („Standard"). Als solche Vergleichslösung wählten wir nach einer Reihe von Vorversuchen (WINNER, 1957) eine 0,01 %ige Lösung von Rivanol (3,9 - Diamino - 7 - äthoxyacridin), da diese Lösung — im Gegensatz zu Wurzelablaufwasser — genau reproduzierbar und über längere Zeit haltbar ist. Als Vergleichsgrundlage für verschiedenzeitige Versuche wurde der Schlüpfeffekt definiert, den eine 0,01 %ige wässrige Lösung von Rivanol bei einer Temperatur von 24°C und einer Einwirkungszeit von 48 Std. bei einer bestimmten Anzahl (3 × 20) Zysten der betreffenden Untersuchungsreihe auslöst. Diese Werte dienten ausschliesslich der Kennzeichnung der Schlüpfbereitschaft der zu verschiedenen Zeiten verwendeten Zysten (vgl. WINNER, 1956) und werden bei den in dieser Arbeit aufgeführten Versuchsergebnissen nicht gesondert angegeben.

Von allen zu prüfenden Exsudaten und ihren Fraktionen wurden Verdünnungsreihen hergestellt, um beim biologischen Test den bekannten Umschlag vom positiven Reiz zum Hemmeffekt auszuschalten. Hierdurch konnten dann Schlüsse auf die Quantität wirksamer Substanz in den untersuchten Mustern gezogen werden. Für den Einzeltest diente als Mass die Summe aller vom Beginn des Schlüpfreizes bis zu seinem sichtbaren Abklingen geschlüpften Nematoden (RE = Reizeffekt).

UNTERSUCHUNGEN AN WURZELEXSUDATEN

1. *Gewinnung der Exsudate*

Für die spezielle Fragestellung dieser Arbeit erschien es zweckmäs-

sig, Wurzelexsudate durch eine kurzfristige Kultur der Pflanzen in destilliertem Wasser zu gewinnen. Wir gingen dabei von dem Gedanken aus, eine möglichst grosse Anzahl Wurzeln junger Keimpflanzen in eine möglichst geringe Menge Wasser hineinwachsen zu lassen. Die von den Spitzen der Wurzeln in das Wasser diffundierenden stickstoffhaltigen Substanzen und polymeren Schleimstoffe verändern dann den osmotischen und physiologischen Wert des destillierten Wassers soweit, dass den Wurzeln in diesem Medium ein begrenztes Weiterwachsen möglich ist. Im einzelnen wurde folgendermassen verfahren:

Gereinigter, vorgequollener Rübsensamen wird auf einem in einen Rahmen gespannten Aluminiumdrahtgeflecht (lichte Maschenweite 1,2 mm) ausgebreitet und mit sauberem, angefeuchteten Filterpapier bedeckt. Der Rahmen wird dann in eine Kunststoffschale eingehängt, deren Boden etwa 0,5 cm hoch mit destilliertem Wasser bedeckt ist. Der Abstand zwischen Maschendraht und Wasseroberfläche beträgt etwa 0,5 cm. Das Papier wird zur Feuchthaltung mit einer Ecke in ein kleines auf den Rahmen gestelltes Wasserschälchen eingetaucht, und die ganze Kunststoffschale wird schliesslich mit einer dicht aufliegenden Glasplatte abgedeckt. — Die Keimwurzeln wachsen nach zwei bis drei Tagen (bei 18 — 22°C) in das Wasser. Sobald die Keimblätter sich aus der Samenschale befreit haben und zu assimilieren beginnen, wird das aufliegende Filterpapier abgenommen. Die in das Wasser hineinwachsenden Wurzeln sind zwar in ihrem Wachstum etwas gehemmt, bleiben aber gesund. Dass bei diesem Verfahren einige Keimwurzeln von Pflanzen, deren Keimblätter es nicht gelingt, die Samenschale vor Abnahme des Filterpapieres zu sprengen, am dritten Tage absterben, wurde mit in Kauf genommen, weil erwartet werden konnte, dass die sich daraus ergebende Veränderung in der Zusammensetzung der Exsudate für die Aufarbeitung zunächst keine besondere Bedeutung haben würde. Gerechnet von dem Zeitpunkt, an dem die Mehrzahl der Wurzelspitzen die Wasseroberfläche durchstossen hat, werden die Pflanzen für 72 Std. in dem Wasser belassen (ca. 1000-1500 in einer Schale mit der Grundfläche 24 × 30 cm). Das so gewonnene „Wurzeldiffusat" wird dann abgegossen, einmal grob filtriert und entweder im Schlüpfversuch verwendet oder weiter aufgearbeitet.

2. *Anreicherung der wirksamen Substanz*

Das Wurzeldiffusat diente zunächst einigen orientierenden Untersuchungen über die Beständigkeit des Reizfaktors.

Diese erbrachten folgende Ergebnisse:

a) Der reizwirksame Anteil insteriler Wurzeldiffusate wird in wenigen Wochen durch Mikroorganismen abgebaut, während er in sterilem Wurzeldiffusat über viele Wochen erhalten bleibt (Aufbewahrung bei Zimmertemperatur).

b) Die Wirksamkeit wird durch Erhitzung des Wurzeldiffusats bis auf 100°C kaum abgeschwächt. Erst nach einer Erhitzung über 100°C im Dampftopf wird die Wirksamkeit merklich herabgesetzt.

c) Durch kurzzeitige Änderungen des pH-Wertes bis zu pH 1 und 12 wird die Aktivität des Wurzeldiffusates nicht aufgehoben. Auch nach einer Eiweissfällung mit $CuSO_4$ bleibt die Wirksamkeit erhalten.

d) Eine völlige Inaktivierung des Wurzeldiffusates konnte nur durch längere Einwirkung von $KMnO_4$ erreicht werden.

Das in den Exsudaten enthaltene wirksame Prinzip ist bei Verwendung einer Cellophan-Membran dialysierbar. Versuche, die wirksame Substanz mit Hilfe von Kunstharzaustauschern (Amberlite, JR - 4 B; Dowex 2, Dowex 50) von störenden Begleitstoffen zu reinigen, zeigten, dass eine Anreicherung und Trennung der wirksamen Komponenten an der Austauchersäule wohl möglich sein dürfte; jedoch ist eine einfache Reinigung der wirksamen Fraktion durch Passage über Ionenaustauscher nicht ohne weiteres zu erreichen, da sowohl vom Anionen — wie vom Kationenaustauscher ein erheblicher Teil des wirksamen Prinzips festgehalten wird.

Dagegen führte ein einfacher Aufarbeitungsgang zur Anreicherung des wirksamen Anteils; dieser umfasste im Einzelnen folgende Schritte, die durch Schlüpfversuche wiederholt kontrolliert wurden:

1. Eindampfen des Wurzeldiffusates von ca 5000 Pflanzen auf 100-fache Konzentration (18 Torr, 35°C).

2. Zentrifugieren des ungereinigten Konzentrates (10 Min., 10 000 Umdr./Min) und Filtration des Überstandes durch groben Membranfilter (Nr. 2).

3. Eindampfen (wie unter 1) bis zur Trockene und Aufnahme des lufttrockenen Rückstandes (8-12 mg) in 1 cm Wasser.

4. Filtration durch feinen Membranfilter (Nr. 1) und Einfrieren des Filtrates bei — 15°C bis zur weiteren Analyse.

Das gereinigte Konzentrat, eine klare Flüssigkeit von schwach gelblicher Färbung, zeichnet sich durch hohe Wirksamkeit aus und löst bei schlüpfbereiten Zysten noch in einer Verdünnung von 1 : 10 000 bis 1 : 100 000 einen deutlichen Schlüpfeffekt aus (Abb. 1).

Aus der Abb. 1 lässt sich erkennen, dass das gereinigte Konzentrat durch die Aufarbeitung im Vergleich zum Ausgangsmaterial nur

wenig an Wirksamkeit eingebüsst hat. Wird das Konzentrat nur schwach verdünnt, wirkt es im biologischen Test hemmend auf den Schlüpfvorgang (Abb. 2). Das entspricht im Wesentlichen den Ergeb-

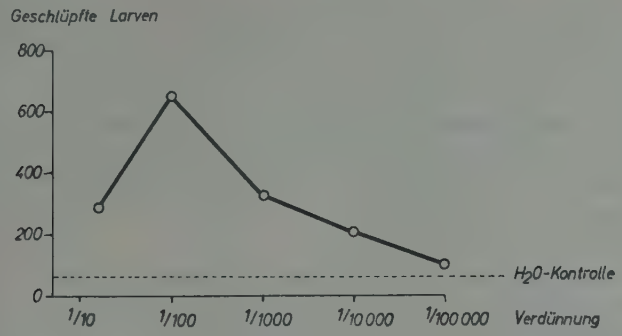

Abb. 1: Wirksamkeit des gereinigten Konzentrates bei zunehmender Verdünnung.

nissen, die FENWICK (1957) und HAGUE (1954) bei ihren Untersuchungen an Wurzeldiffusaten der Kartoffel und deren Wirkung auf den Kartoffelnematoden (*Heterodera rostochiensis*) erarbeitet haben.

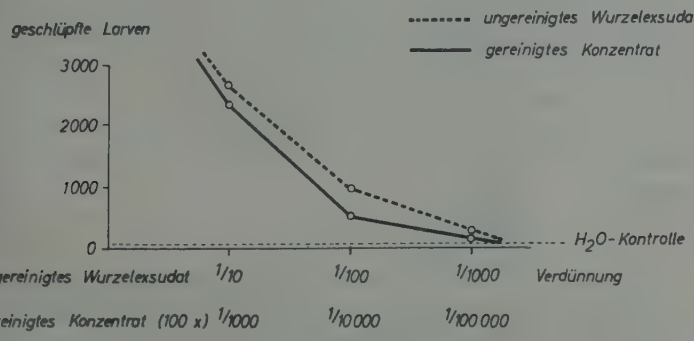

Abb. 2: Wirksamkeit des gereinigten Konzentrates im Vergleich zum ungereinigten Wurzelexsudat bei äquivalenter Verdünnung.

3. *Versuche zur Isolierung der wirksamen Fraktionen*

a) Papierchromatographische Trennung

Unter Verwendung von Papiersorte Schleicher u. Schüll 2043 b wurde überwiegend im aufsteigenden Verfahren gearbeitet. In einigen Versuchen wurde auch die Papiersorte 2045 a verwendet bezw. im absteigenden Verfahren gearbeitet. Die Arbeitstemperatur betrug 24 ±

1°C. Folgende Entwicklungsgemische erwiesen sich als geeignet:

1. Methyläthylketon, wassergesättigt
2. s-Butanol: Ameisensäure: Wasser (75 : 13 : 12)

Die am Startpunkt auf das Papier aufgetragene Menge des gereinigten Konzentrates (20-40 cmm) richtete sich nach der Aufnahmefähigkeit des Papieres. Die entwickelten Chromatogramme wurden nach dem Trocknen über die Länge der Laufstrecke (25 cm) in Segmente zerschnitten. Das Segment, durch dessen Mitte die Startlinie läuft, erhielt die Nr. 1, die übrigen Segmente wurden fortlaufend nummeriert (vgl. Abb. 3-5). Die einzelnen, meist 2 cm breiten Papierstreifen wurden getrennt mit Wasser eluiert, und die Eluate nach Verdünnung im Schlüpfversuch ausgetestet.

Da auch schwach verdünnte Eluate von Papierstreifen ohne aufgetragene Substanz — je nach Papiersorte und Zusammensetzung des Lösungsmittelgemisches — einen Schlüpfreiz induzieren können, wurde in die Versuche als weitere Bezugsgrösse ein Versuchsglied aufgenommen, in dem die Wirkung vom Eluat eines leeren Papierstreifens getestet wurde, des ausser den Rückständen des Lösungsmittels keine aufgetragenen Substanzen enthielt. Bei den folgenden Diagrammen, in denen die Ergebnisse mehrerer verschiedener Versuche mit Chromatogrammfraktionen zusammengefasst sind, wurde als Bezugsgrösse der Reizeffekt zugrunde gelegt, der jeweils vom Eluat eines solchen leeren Papierstreifens (p - Eluat) ausgelöst wurde. Die Anzahl der im p - Eluat geschlüpften Nematoden wurde gleich 1 gesetzt, und die Anzahl der in den übrigen Versuchsgliedern geschlüpften Nematoden darauf bezogen. Den auf das p-Eluat bezogenen Reizeffekt haben wir als REp bezeichnet,

$$\text{REp} = \frac{\text{Geschlüpfte Larven}}{\text{Geschlüpfte Larven im Eluat des leeren Papieres}}$$

Unter Berücksichtigung der bestehenden Arbeitsmöglichkeiten wurden die Eluate meist nur in solcher Verdünnung für den biologischen Test verwendet, bei der irgendwelche Hemmwirkungen ausgeschlossen waren, die Fraktion mit der stärkeren positiven Wirkung sich aber noch deutlich aus der Reihe der übrigen Fraktionen heraushob.

In Abb. 3 und 4 sind die Ergebnisse der verschiedenen Versuche zur papierchromatischen Trennung der Komponenten mit den Lösungsmittelgemischen I und II zusammengefasst (Mitlaufende Vergleichssubstanzen: Lösungsmittelgem. I → Aesculin, Rf-Wert 0,27; Lösungsmittelgem. II → Leucin, Rf-Wert 0,63).

Aus den Diagrammen ist zu ersehen, dass es mit den verwendeten Lösungsmittelgemischen möglich ist, eine stärkstwirksame Fraktion

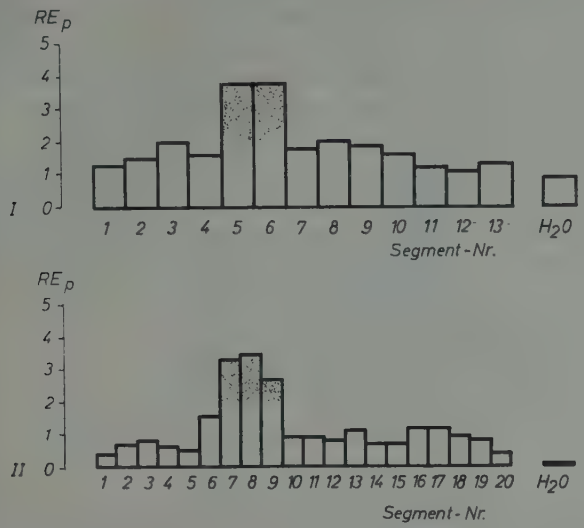

Abb. 3: Verteilungsdiagramme nach papierchromatographischer Fraktionierung des gereinigten Konzentrates mit Lösungsmittelgemisch I (Mittel von 5 Versuchen). — Beim Diagramm II betrug die Breite des Segmente nur 1,7 cm (Laufstrecke 34 cm).

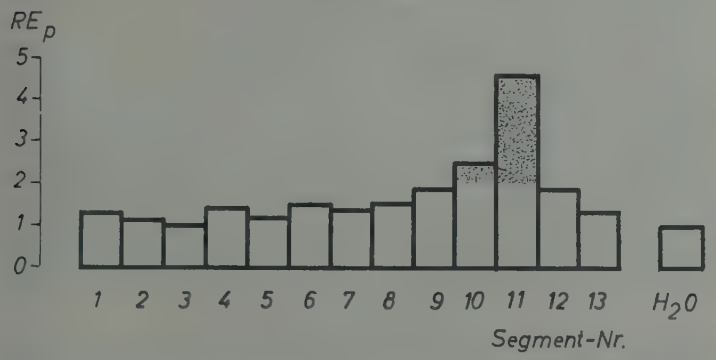

Abb. 4: Verteilungsdiagramm nach papierchromatographischer Fraktionierung des gereinigten Konzentrates mit Lösungsmittelgemisch II (Mittel von 6 Versuchen).

der Exsudate auf einer deutlich ausgeprägten Vorzugszone von den übrigen mehr oder weniger unwirksamen Fraktionen zu trennen. Be-

sonders scharf ausgeprägt war die Vorzugszone bei Verwendung von Lösungsmittelgemisch II, wo die stärkst wirksame Komponente mit einem Rf-Wert von etwa 0,8 in Erscheinung tritt.

Auf Grund dieser Ergebnisse versuchten wir, die in den Vorzugszonen lokalisierten Substanzen durch zahlreiche empfindliche Gruppenreagenzien färberisch sichtbar zu machen (Reaktion auf Zucker, Zukkeralkohole, Aminosäuren, Proteine, organische Säuren, Aldehyde, Phenole, Indole, Alkaloide, Purine, Flavone). Keine einzige der zahlreichen Substanzen, die durch eine Farbreaktion sichtbar gemacht werden konnten, lag nach Chromatographie mit dem Lösungsmittelgemisch II innerhalb der Vorzugszone (Lösungsmittelgem. II: Segm. Nr. 11). Fast alle reaktiven Substanzen wiesen einen Rf-Wert $< 0{,}7$ auf und kamen daher als stärkst wirksame Komponente nicht in Frage.

b) Elektrophoretische Trennung

Neben der papierchromatographischen Arbeit wurde eine Isolierung der wirksamen Substanz mit Hilfe der Elektrophorese versucht. Wir verwendeten dazu ein Horizontal-Elektrophorese-Gerät nach Fuhrmann, das im wesentlichen der von Wieland und Fischer (1948) beschriebenen Apparatur entspricht (Elektrophorese bei pH 6, 150 V, Laufzeit 3 Std.). Was bei einigen vorausgegangenen Versuchen (Winner, 1956) sich schon hatte erkennen lassen, konnte nach elektrophoretischer Trennung der Substanzgemische und Prüfung der Fraktionen im biologischen Test bestätigt werden: Die aktivierende Wirkung ist nicht nur an eine einzige Komponente gebunden. Sowohl Substanzen ohne Eigenladung wie auch ladungspositive und ladungsnegative Bestandteile üben in bestimmter Konzentration einen Schlüpfreiz aus.

Unterwirft man nur die Fraktion der Vorzugszone, die sich nach Papierchromatographie mit Lösungsmittelgemisch II ausbildet, einer anschliessenden Elektrophorese, lassen sich von einer besonders wirksamen unpolaren Komponente noch aktivierend wirkende Begleitsubstanzen trennen, die die Wirkung der Hauptkomponente verstärken.

UNTERSUCHUNGEN AN WURZELEXTRAKTEN

Das Misslingen eines Farbnachweises der am stärksten wirksamen Komponente konnte möglicherweise darin begründet liegen, dass die Konzentration des fraglichen Stoffes immer noch zu gering war, um bei einer Farbreaktion überhaupt in Erscheinung zu treten. Es schien daher geraten, die betreffende Substanz in grösseren Mengen zu ge-

winnen, als das auf dem Wege über Wurzel a u s s c h e i d u n g e n in der von uns entwickelten Methode möglich ist.

Es wurde deshalb geprüft, ob sich bei gleichem Trennungsgang auch aus Wurzel e x t r a k t e n wirksame Fraktionen gewinnen lassen, die den stärkst wirksamen Fraktionen der Wurzelexsudate entsprechen.

Es wurden Rübsenpflanzen auf Aluminiummaschendraht angezogen (s.o.) und acht Tage in einer 1/10 verdünnten Nährlösung (nach Tol-

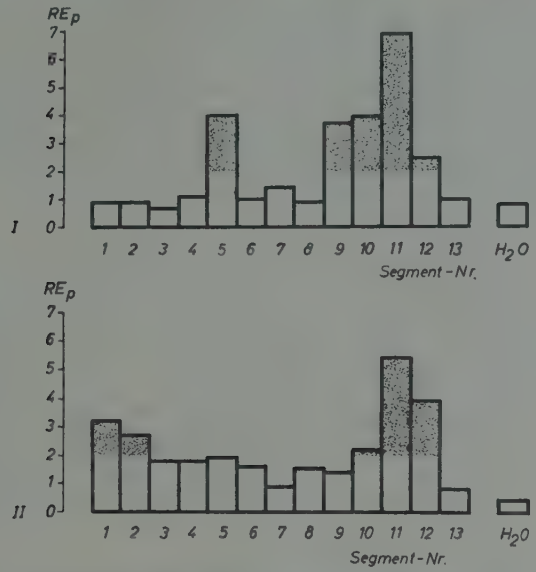

Abb. 5: Verteilungsdiagramme nach papierchromatographischer Fraktionierung von Wurzel*extrakten* mit Lösungsmittelgemisch II. Diagramm I: Wässriger Extrakt der Wurzeln. Diagramm II: Aceton - Extrakt der Wurzeln.

lens) wachsen gelassen. Die Wurzeln wurden dann gründlich abgespült, abgeschnitten und extrahiert. Die Extraktionsmittel (Wasser, Aceton, Äthanol) wurden dann abgedampft und die Rückstände zu gleichen Teilen in Wasser aufgenommen. Die Aufarbeitung erfolgte in der gleichen Weise (s.o.) wie bei den Wurzelexsudaten.

Sowohl beim wässerigen wie beim acetonigen Extrakt der Wurzeln wurde nach Chromatographie mit Lösungsmittelgemisch II eine stärkst wirksame Fraktion isoliert, deren Lage auf dem Papier einen Rf-Wert von etwa 0,8 entspricht (Abb. 5).

Das bedeutete eine auffallende Übereinstimmung mit der Vorzugs-

zone, die nach chromatographischer Trennung der Wurzel e x s u d a t e ermittelt worden war (Abb. 4). Ausserdem lässt sich erkennen, dass in den Extrakten der Wurzeln reizwirksame Stoffe enthalten sind, die in den Exsudaten entweder nur in sehr geringer Menge oder überhaupt nicht auftreten (vgl. die Komponente im wässrigen Auszug mit einem Rf-Wert von etwa 0,3). Jedoch ist die Zusammensetzung der Extrakte im Hinblick auf reizwirksame Substanzen je nach dem verwendeten Lösungsmittel unterschiedlich, wie auch vergleichende elektrophoretische Untersuchungen uns bestätigten.

Versucht man überschläglich die Wirksamkeit der Exsudate und Extrakte zu vergleichen, so lässt sich unter Berücksichtigung der Vergleichsgrössen (Rivanol-Test) nachweisen, dass die wirksamen Substanzen in den Extrakten — bezogen auf die gleiche Anzahl Wurzeln — in wesentlich höherer Konzentration vorliegen als in den Exsudaten.

DISKUSSION

Die chromatographischen und elektrophoretischen Untersuchungen zeigen, dass in den Wurzelexsudaten des Rübsens verschiedene, deutlich unterscheidbare Substanzen einen Schlüpfreiz ausüben. Da die aktivierende Wirkung des unfraktionierten Exsudates stärker ist als jeder Reiz, der von irgend einer Teilfraktion ausgeht, muss man annehmen, dass der starke Reiz der Wurzelausscheidungen auf dem Zusammenwirken verschiedener Stoffe beruht. Die diskontinuierliche Wirkungsabnahme bei partieller Zerstörung der organischen Substanz durch physikalische oder chemische Einwirkung (s.o.) fände damit zum Teil eine Erklärung.

Eine Identifizierung der am stärksten reizwirksamen Komponente war bisher noch nicht im positiven Sinne möglich. Die negativen Befunde ermöglichen aber, eine Reihe von Stoffen aus weiteren Überlegungen auszuschliessen. So handelt es sich bei der stärkst wirksamen Komponente zweifellos nicht um eine der bisher aus der Natur bekannten Aminosäuren, da die innerhalb dieser Gruppe im Butanolgemisch am weitesten laufende Aminosäure Leucin, die als Vergleichssubstanz diente, nur einen Rf-Wert von 0,63 erreicht. Dieses Ergebnis erscheint uns besonders wichtig, da speziell für die Gruppe der Aminosäuren eine natürliche Ausscheidung durch die Wurzeln mehrfach nachgewiesen ist (vgl. Huber u. Bauer, 1955; Katznelson et al., 1955) und mit einigen reinen Aminosäuren enzystierte Larven zum Schlüpfen angeregt werden können (Wallace, 1956).

Die Vermutung, dass insbesondere dem Alanin (Rensch, 1925)

oder der Glutaminsäure (Wood u. Serro, 1954) in den Wurzelexsudaten von Wirtspflanzen eine besondere Bedeutung für die Aktivierung von *Heterodera schachtii* zukommt, trifft daher zumindest für junge Rübsenpflanzen nicht zu. — Auch eine Reihe anderer organischer Verbindungen kommen mit Sicherheit als stärkst wirksame Komponente nicht mehr in Betracht (Winner, 1956), worauf hier jedoch im einzelnen nicht näher eingegangen werden kann.

Da auch nach chromatographischer Trennung des Acetonextraktes (Lösungsmittelgemisch II) eine deutliche Vorzugszone mit einem Rf-Wert von etwa 0,8 auftritt, und da schliesslich auch der wirksame Anteil der Wurzelexsudate in Aceton löslich ist, kann man annehmen, dass es sich sowohl beim Wurzelextrakt wie auch bei dem Wurzelexsudat um die gleiche stärkst-wirksame Komponente handelt. Die Ausbildung der zweiten ausgeprägten Vorzugszone im Wirkungsdiagramm des Acetonauszuges deutet darauf hin, dass in der Wurzel noch andere aktivierend wirkende Stoffe enthalten sind, die in den Exsudaten nicht in erfassbarer Menge vorkommen.

Herrn Prof. Dr. W. H. Fuchs, Institut für Pflanzenpathologie - Göttingen, danke ich für seine Hilfe und seinen Rat bei Planung und Durchführung der Arbeit. Ebenfalls danke ich Herrn Prof. Dr. H. Lüdecke für seine Unterstützung und für einen Arbeitsplatz im Göttinger Institut für Zuckerrübenforschung.

SUMMARY

By culturing seedling-plants of turnip rape [*Brassica rapa oleifera*] in destilled water, root-exudates were collected and investigated for their activity to stimulate hatching of encysted larvae of *Heterodera schachtii*.

It was established that the active principle of the exudates can be partially destroyed by various physical and chemical means as well as by bacterial degradation. Total loss of the active principle can only be achieved by rather complete destruction of the organic material contained in the root-exudates.

The active principle is dialysable and is retained by ionic exchange resins in a varying degree depending on the nature of the resin. Careful concentration of the exudates to dryness in vacuo at 35°C does not appreciably affect the activity of the material.

Paper electrophoresis of the root-exudates shows that the active principle can be separated into various, in part polar, compounds. The active components can also be separated by means of paperchromatography. The relative position of the compounds in question on the paper chromatogram gives some indication on the probable nature of these substances. A very distinct zone of activity on the paper can be obtained by using s-Butanol: Formic-acid: Water (75 : 13 : 12) as a solvent system, in which the component with the highest activity migrates with a Rf-value of appr. 0.8.

It was not yet possible to find a colour reaction for the most active component

on the paper. This indicates that the concentration of the most active component on the paper was still to low or that the component in question does not react with any of the staining reagents tested.

Further experiments showed however, that the most active substance can not only be found in root-exudates but also, and in much higher concentration, in the roots of young seedlings from which it can be extracted without difficulty.

With the technics already applied, further analytical investigations of the hatching factors in *Brassica rapa* seem to be promising.

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Eingegangen: 24. April 1958

Nematologica III (1958) : 327-331. E. J. Brill, Leiden

ROTYLENCHOIDES BREVIS N.G., N.SP.

(Rotylenchoidinae n. subfam. : Tylenchida)

BY

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The roots of a cultivated banana plant (*Musa paradisiaca* L. v. *sapientum*) from Mbuzii in the Lushoto district of the West Usambara Mountains, Tanganyika Territory were found to be fairly heavily attacked by species of *Pratylenchus* Filipjev, 1934, and a species of a new genus, hereby named *Rotylenchoides* n.g. Roots affected by the type species *R. brevis* n.g., n.sp., when placed in closed jars with a little water, yielded large numbers of the nematode, which subsequent dissection of the roots showed to be an endoparasite of the root cortex. Nothing is known of the pathogenicity of *R. brevis* and to date the attacks have not been found outside the Lushoto district. *R. brevis* is placed in a new subfamily Rotylenchoidinae of the family Tylenchidae Filipjev, 1934, to receive genera with characters intermediate between those of the subfamilies Hoplolaiminae Filipjev, 1934 (emend. Thorne, 1949) and Pratylenchinae Thorne, 1949.

ROTYLENCHOIDINAE N. SUBFAM.

Diagnosis. Tylenchidae. — Head with six lips, annulated, not offset by constriction; head framework strongly developed; body cuticle striated, lateral field marked by incisures; spear long, strong, with well developed basal knobs, oesophagus with ovate, valvate median bulb and lobelike posterior oesophageal region, extending over anterior end of intestine; phasmids at about level of anus; female with single prodelphic ovary, vulva posterior; tails of both sexes not longer than anal body diameter, male tail enveloped by bursa; gubernaculum present; a telamon may be present. (Type genus : — *Rotylenchoides* n.g. with characters as set out above).

Type species : *Rotylenchoides brevis* n.g., n.sp.

Dimensions : —

6 ♀♀ : L = 0.43-0.53 mm; a = 18-22; b = 3.6-4.4;
c = 36-55; V = 89.7-92.1 %; Spear length = 26-29 μ

11 ♂♂: L = 0.37-0.44 mm; a = 24-30; b = 3.4-4.3;
c = 23-36; Spicule length = 21-25 μ
Gubernaculum = 4-8 μ; length of bursa = 25-31 μ;
Telamon length = 11 μ (1 specimen).

Holotype ♀: L = 0.49 mm; a = 21.4; b = 3.7; c = 54.8; V = 92.1%

Allotype ♂: L = 0.42 mm; a = 30; b = 3.9; c = 30;

Head a truncated cone with 3 sometimes 4 annules. Head framework strongly developed consisting of a basal plate, extending back into the sides of the body for 3 annules, a strongly developed skeletal tube arising from the middle of the basal plate and surrounding the spear tip, and a thin skeletal shield arising distally from this, supporting the lips and sides of the head. There is no constriction between the head and the rest of the body, but the three intervening annules are very narrow.

From the middle of the basal plate a thick cylindrical spear guide surrounds the fore part of the spear. Spear is stout and about 3 head widths long. Spear shaft slightly shorter than tapering fore part and stout spear knobs are flattened anteriorly.

Precorpus long; valvular median bulb ovate; isthmus short; oesophageal glands in a lobe of varying shape overlapping the anterior portion of the intestine, which appears to join the oesophagus behind the nerve ring. Nuclei of oesophageal glands not observed. Orifice of dorsal oesophageal gland slightly less than $1/3$ spear length behind spear base in female, not observed in male. Excretory pore opposite middle of glandular lobe, traces of hemizonid immediately anterior in male.

Intestine well supplied with fat granules. Rectum thinwalled, anus obscure in most specimens examined. Ovary short with two rows of oogonia. Uterus longer than gonad and a rounded spermatheca lies near its proximal end. Vagina reaches just beyond half body width, vulva at about 90 % of body length. Postvulval uterine sac present, about one vulval body width long. Testis with double row of spermatocytes passing into a longer vas deferens, full of spermatozoa. Walls of vas deferens are composed of large squamous cells. Narrow ejaculatory duct passes between spicules to cloaca. Bursa arises just anterior to spicule heads and surrounds tail tip. Spicules have slightly expanded, open heads and strongly developed blades. Gubernaculum is very thin. A telamon was observed between the spicules in a single specimen, in which the spicules were not extended on fixation. Lateral field arises some six annules behind head. At about the fourteenth annule a median

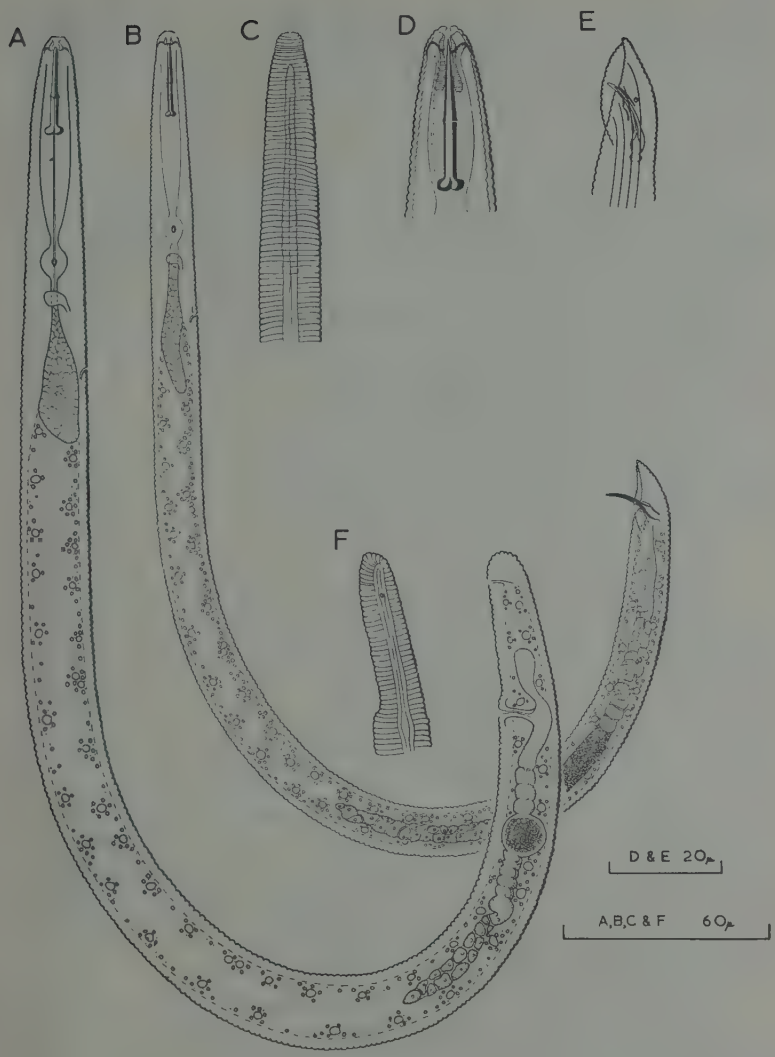

Fig. 1. A. General illustration of female *Rotylenchoides brevis* n.g., n.sp. — B. General illustration of male *R. brevis*. — C. Lateral view of cuticle of head and anterior end of body, showing origin of lateral fields of female worm. — D. Detailed illustration of female head. — E. Tail region of male. — F. Lateral view of cuticle of female posterior body region.

incisure divides the field into two bands which are cross-striated and which extent to a point just behind the median bulb of the oesophagus, where the median incisure bifurcates. Striation of the lateral field ceases here and three plain bands extend down the body. In female, lateral field is somewhat expanded at level of vulva and ends just before tail tip (Fig. 1F). In male the field expands over base of bursa (Fig. 1E).

Phasmids small and situated at about anal level; in the middle of the lateral field in female, while those of male are situated either in middle of lateral field or between dorsal and dorso-lateral incisures (Fig. 1E).

Type host. Roots of *Musa paradisiaca* L. var. *sapientum*, cultivated banana.

Type locality. Mbuzii, Lushoto District, W. Usambara Mts., Tanganyika Territory.

Type specimens. Holotype ♀ No. 11 M.s. 1, Allotype ♂ No. 6 M.s. 1, in collection of Nematology Department, East African Agriculture and Forestry Research Organisation, P.O. Box 21, Kikuyu, Kenya.

Discussion. — *R. brevis* occupies an interesting taxonomic position. In common with Hoplolaiminae the genus *Rotylenchoides* has a heavily sclerotised head framework, a massive spear at least three times as long as the width of the head base, with well developed basal knobs, lobe-like posterior oesophageal region and a tail not longer than the anal body width. However, unlike Hoplolaiminae, *Rotylenchoides* has but a single gonad and a posterior vulva, in which characters the new genus resembles *Pratylenchus*, a genus of the subfamily Pratylenchinae. *Rotylenchoides* differs from Pratylenchinae in that the latter has a spear not more than twice as long as the width of the head base, the tails of the vermiform species are at least twice the length of the anal body diameter, the phasmids are located behind the level of the anus and the median bulb is spheroid.

To include *Rotylenchoides* with Hoplolaiminae it would be necessary to emend the diagnosis of the subfamily as regards the possession of two ovaries and a vulva at the middle of the body, in this group. In view of the fact that the subfamily Hoplolaiminae as it stands at present, constitutes a compact group in the present classification of the order *Tylenchida* Thorne, 1949, it is felt that separation of *Rotylenchoides* to a new subfamily is justified.

ACKNOWLEDGEMENTS

The author is indebted to Mr. F. G. W. Jones and Dr. J. Basil Goodey of the Nematology Department, Rothamsted Experimental Station, England, for valued criticism of the text.

RESUMÉ

L'auteur donne une description de *Rotylenchoides brevis* n.g., n.sp. (Rotylenchoidinae n. subfam.: Tylenchida).

Les Rotylenchoidinae diffèrent des *Hoplolaiminae* par le fait qu'ils possèdent un ovaire simple et une vulve postérieure et des Pratylenchinae par leur stylet mesurant au moins trois fois la largeur de la tête, l'emplacement des phasmides au niveau de l'anus, une queue plus courte que celle des espèces vermiformes des Pratylenchinae et par la forme ovale du bulbe aesophagien central.

REFERENCE

THORNE, G. (1949), On the classification of the *Tylenchida,* new order (Nematoda, Phasmidia). *Proc. helm. Soc. Wash.* **16**: 37-73.

Received for publication: April 28, 1958

Nematologica III (1958) : 332-349. E. J. Brill, Leiden

ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) IN THE FEDERATION OF RHODESIA AND NYASALAND

BY

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Very brief lists of plant hosts for *Meloidogyne* spp. in the Federation under the earlier names *Heterodera marioni* or *H. radicicola*, were published by SMEE (1923, 1928) and COLLINS (1937, 1938) but it was not until the work by JACK (1943) that any serious attempt was made to compile a comprehensive list. Additions and alterations to this list were made by JACK (1944, 1945).

MARTIN (1953) published a list of all the known host records for the Federation and in addition included plants which were known to be or likely to be grown in the Federation and had elsewhere been recorded as hosts.

As a result of work by CHITWOOD (1949) investigations were started in 1953 to ascertain the species of *Meloidogyne* present in the Federation and the first host list along these lines was produced by MARTIN (1955). These researches revealed that at least three of the known species of *Meloidogyne* were present, *M. javanica* (Treub 1885), *M. incognita var. acrita* Chitwood, 1949, and *M. hapla* Chitwood, 1949. No information was given on species distribution. Since that date, a further species *M. arenaria* Chitwood, 1949, has been discovered in the Federation and very many more records of host plants have been collected.

MARTIN (1956-1957) published accounts of the host-parasite relationship between eighteen different crops of economic importance and *M. javanica*. This work showed that differences apparently exist between the race or strain of *M. javanica* found in Southern Rhodesia and that occurring in Maryland, U.S.A. SASSER (1954) states that peanuts and cotton are resistant to *M. javanica*; in S. Rhodesia they were found to

Fig. 1. Outline map of the Federation showing the known distribution of *Meloidogyne javanica* (shaded areas)

be capable of acting as hosts and were rated as lightly susceptible. This paper is restricted to presenting all the known records (for the Federation) of host plants wherein the species of *Meloidogyne* have been determined. Data are also given on frequency of occurrence and the known distribution. Most of the records are of naturally occurring infections and have been compiled from specimens either collected during expeditions by the author or from plants received from farmers, gardeners, and other interested persons. A few records were obtained from museum specimens preserved in the Entomological Branch at Salisbury and the Department of Agriculture, Zomba, Nyasaland. It was noteworthy to find that one of the earliest known infected tobacco plants, a specimen collected by R. W. Jack at Salisbury in 1912, was attacked by *M. incognita var. acrita*.

M. javanica is to be found to a greater or lesser degree in many of our cultivated lands, especially in Mashonaland, and is considered to be indigenous. On record are a number of instances of quite heavy infections being found in plants growing in apparently virgin situations. *M. incognita var. acrita* has been discovered on three occasions in agricultural farm lands, in each case in association with naturally moist soil conditions. These two species of *Meloidogyne* are the only ones so far discovered in what can be truly described as farm lands or fields. *M. hapla, M. javanica* and *M. incognita var. acrita,* are commonly recorded from market gardens (particularly where vegetables are grown amongst fruit trees imported from South Africa), flower and vegetable gardens in both town and country districts, and intensive irrigation schemes. *M. arenaria* has been only recently discovered and is known from relatively few areas as yet, but has not been found infesting farm lands.

Seed potato crops have been found infected with the four recorded *Meloidogyne* species, but only light, naturally occurring infections of *M. hapla* have been found and all of these have been confined to the roots and have not affected the tubers. The dissemination of infected seed tubers is probably one of the main avenues of spread of *M. incognita var. acrita* and may be expected to assist in the future spread of *M. arenaria.*

Fairly heavy root infections of *M. hapla* have been produced experimentally, using the potato variety "Tedria", but in no case have the tubers exhibited any macroscopic evidence of infection. A few mature females with large egg masses have, however, been found at the heel ends of tubers at two different sites, where the stolon is attached and

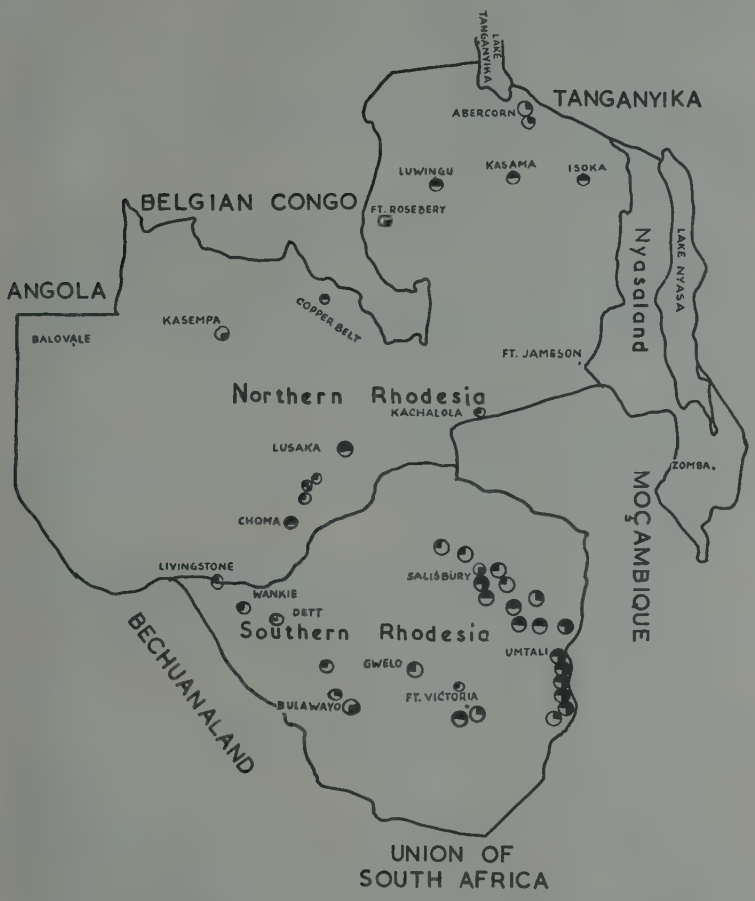

Fig. 2. Outline of the Federation showing the known distribution of:

- *Meloidogyne incognita* var. *acrita*
- *M. hapla*
- *M. arenaria*

at the point of emergence of small basal rootlets. Whether or not the parasites are sufficiently embedded in the tubers to avoid desiccation during storage is as yet unknown.

It would seem that wherever there is a history of extensive introductions of plants, roots, tubers or soil it is usual to find at least two of the four recorded species of *Meloidogyne* but where no such history is known, then only *M. javanica* is found.

RESPONSE AND HOST VALUE SYMBOLS

In view of the wide variation observed between plant species in the degree of galling, numbers of female nematodes and abundance of egg masses produced, it is considered desirable to present observations which may indicate normal relationships between plant and root-knot nematode species.

Throughout the period covered by the examination of several thousand root systems, records have been kept of the approximate numbers and types of galls and swellings seen at each examination, together with data on the number of egg masses and apparently mature females present.

In a number of instances the degree of galling appeared excessive when compared with the number of developed females or egg masses seen. This may not be of any significance if only a single or few specimens from one or two sites have been examined but if constant when many specimens from a number of sites have been seen, then it would appear indicative of a normal relationship between the parasite and infected plants.

A guide to the numbers of specimens and sites concerned will be found in Table I. The response and host value figures given in Table I, 2nd column, are presented either as single numbers not enclosed by brackets, in which case the abundance of egg masses observed seemed to be directly correlated with the degree of galling, or where there are two numbers, one of which is enclosed by brackets, the first refers to the degree of galling and the second (in brackets) to the abundance of egg masses. The latter is only given where the direct correlation was not apparent.

It must be understood that root-knot nematodes are known to occur over a much wider area than is depicted in the distribution maps (figs 1 and 2), but identification of the species of *Meloidogyne* involved has not yet been determined.

Table 1

Plant species attacked by root-knot nematodes (Meloidogyne spp.) in the Federation of Rhodesia and Nyasaland.

SECOND COLUMN

Figures not enclosed by brackets

0 = No galls visible to the naked eye.
1 = Only one or two small swellings present.
2 = Few swellings present.
3 = Swellings or galls readily found.
4 = Swelling or galls visible at a glance.
5 = Numerous galls or swellings present.
6 = Roots almost totally galled or covered with swellings.

Figures enclosed by brackets

(0) = No egg masses seen.
(1) = Only one or two egg masses present.
(2) = Few egg masses.
(3) = Egg masses readily found.
(4) = Egg masses visible at a glance.
(5) = Egg masses numerous.

THIRD COLUMN

A = Single specimen examined.
B = Two to five specimens examined.
C = Six to ten specimens examined.
D = Eleven to twenty specimens examined.
E = More than twenty specimens examined.

Numbers 1, 2, 3 etc. indicate the actual number of infested sites from which specimens were obtained.

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* Tubers not infected except in the case of variety "Tedria" as explained in the text.

acr. = *Meloidogyne incognita* var. *acrita*
ar. = *M. arenaria*
hap. = *M. hapla*
jav. = *M. javanica*
exp. infs = Experimental infections.

Scientific, common and varietal names of plants.	*Meloidogyne* spp. with response and host values.	No. of observations and sites.
Acacia mollissima Willd., Black Wattle	jav. 6	D. 4
Acalypha segetalis Muell. Arg.	acr. 3	A. 1
Acanthaceae	hap. 3 (1)	A. 1
Acanthospermum hispidum DC.	jav. 1	B. 1
Achyranthes aspera L.	ar. 4 (0), jav. 5	ar. B. 1, jav. B. 2
Agapanthus africanus (Hoffmsg.), African Lily	acr. 2, jav. 3	acr. B. 1, jav. C. 3
Ageratum conyzoides L.	acr. 3, hap. 3	acr. A. 1, hap. C. 1
Alchemilla sp., Lady's Mantle	hap. 3, jav. 6	B. 1
Allium ascalonicum L., Shallot	acr. 4, jav. 4	acr. E. 3, jav. E. 2
Allium cepa L., Onion	acr. 2, ar. 4, jav. 5	acr. C. 2, ar. D. 1, jav. E. 10
Id., var. Cape Flat	acr. 3, ar. 3	B. 1
Allium porrum L., Leek	jav. 4	C. 3
Allium schoenoprasum L., Chive	jav. 4	E. 1
Aloe cameronii Hemsl.	jav. 4	B. 1
Alstonia sp.	acr. 4 (3)	A. 1
Alternanthera repens (L.) Kuntze, Khaki Weed	jav. 5 (1)	B. 1
Althaea rosea (L.) Cav., Hollyhock	jav. 3	B. 1

TABLE I (*continued*). See legend on page 337

Alysicarpus rugosus (Willd.) DC.	jav. 5	A. 1
Amarantus hybridus L.	acr. 5, ar. 1, jav. 4	acr. E. 1, ar. D. 1, jav. D. 1
Amarantus spinosus L.	acr. 2	C. 1
Amarantus thunbergii Moq.	acr. 6, jav. 5	acr. D. 1, jav. E. 9
Amarantus viridis L.	jav. 3	B. 1
Ananas sativus Schult., Pineapple	acr. 1	A. 1
Antigonon leptopus Hook. and Arn., White Coral Creeper	acr. 3	A. 1
Antirrhinum majus L., Snapdragon	hap. 6, jav. 6	hap. C. 3, jav. D. 5
Apium graveolens L. var. *dulce,* Celery	acr. 4, hap. 3, jav. 6	acr. A. 1, hap. B. 1, jav. E. 10
Id. id., id., var. White Plume	jav. 6	E. 1
Arachis hypogaea L., Groundnut, var. Virginia Bunch	ar. 3, jav. 1 (0)	ar. A. 1, jav. B. 1 exp. infs.
Id., var. Virginia Jumbo	jav. 2	E. 2 exp. inf.
Id., var. Natal Common	jav. 2	E. 2 exp. inf.
Asclepias curassavica L.	jav. 5 (1)	B. 2
Aspilia sp.	jav. 3	A. 1
Aster sp., Michaelmas Daisy	hap. 1	B. 1
Avena sativa L., Oats var. S.E.S.	jav. 4	E. 2 exp. infs.
Babiana hypogea Burch.	jav. 1 (0)	A. 1
Begonia sp.	jav. 4	B. 2
Berkheya subulata Harv.	jav. 3	B. 1
Beta vulgaris L. var. *cicla,* Swiss Chard	acr. 3 (1). ar. 6 (2), hap. 3, jav. 4 (3)	acr. C. 2, ar. B. 1. hap. B. 1, jav. D. 5
Beta vulgaris L., Beetroot	acr. 6 (4). jav. 6 (4)	acr. C. 3, jav. E. 9
Id. var. Detroit Red	acr. 4, jav. 4	B. 1
Beta vulgaris L., Beet Spinach	jav. 5	C. 4
Bidens biternata (Lour.) Merr. and Sherff., Blackjack	ar. 2 (0), hap. 0, jav. 4	ar. B. 1, hap. E. 5, jav. E. 3
Bidens pilosa L., Blackjack	hap. 5, jav. 0	hap. E. 30, jav. E. 50
Bidens schimperi Sch. Bip. ex Walp.	hap. 5, jav. 5	hap. B. 1, jav. E. 2
Borreria diodon K. Schum., Rhodesian Forget-me-not	jav. 3	B. 1
Borreria scabra (Schumach. & Thonn.) K Schum., Buttonweed	jav. 5	D. 11
Borreria stricta (L.f.) G. F. W. Mey	acr. 4	B. 1
Borreria sp.	hap. 2, jav. 3	A. 1
Brassica oleracea L. var. *acephala,* Curly Kale or Borocole	ar. 2, jav. 4	B. 1
Brassica oleracea L. var. *botrytis* L., Cauliflower	acr. 4 (2), ar. 3 (2), jav. 3	acr. B. 1, ar. B. 1, jav. B. 2
Brassica oleracea L. var. *capitata* L., Cabbage	acr. 5 (2), ar. 2 (1), jav. 4	acr. B. 1, ar. B. 1, jav. E. 15
Id. var. Early Jersey Wakefield	jav. 4 (0)	A. 1
Id. var. Drumhead	jav. 5	A. 1
Id. var. Spitzkool	jav. 5	B. 2
Brassica oleracea L. var. *capitata* L., Red or Pickling Cabbage	ar. 4, jav. 4	ar. A. 1, jav. B. 2
Brassica oleracea L. var. *gemmifera* Zenker, Brussels Sprout	ar. 2 (1), jav. 5	ar. B. 1, jav. B. 3

TABLE I (*continued*). See legend on page 337

Brassica oleracea L. var. *gongyloides*, Kohlrabi	acr. 3, jav. 3	acr. B. 1, jav. B. 2
Brassica rapa L., Turnip	acr. 4, jav. 6 (3)	acr. A. 1, jav. B. 3
Id. var. Purple Top	hap. 4	B. 1
Buddleia sp.	jav. 3	B. 1
Bulbostylis burchellii (Fic. & Hiern) C. B. Cl., Sedge	acr. 5, jav. 3	B. 1
Cactaceae, Ornamental Cactus spp.	acr. 4, jav. 4	C. 1
Cajanus cajan (L.) Millsp., Dahl or Pigeon Pea	jav. 4	B. 2
Calendula officinalis L., Marigold	ar. 5, jav. 5	ar. B. 1, jav. C 4
Callistephus chinensis Nees, Chinese Aster	hap. 3 (1)	B. 1
Capsicum frutescens L., Red Peper	acr. 4	C. 3
Capsicum frutescens L. var. *longum*, Chili Pepper	acr. 4	A. 1
Cardamine hirsuta L., Bitter Cress	acr. 3	E. 1
Carica papaya L., Pawpaw Melon	acr. 5, ar. 4, jav. 5 (4)	acr. C. 6, ar. B. 1, jav. E. 19
Cassia absus L.	jav. 4	A. 1
Cassia kirkii Oliv.	ar. 4, jav. 3	ar. C. 1, jav. B. 2
Cassia mimosoides L.	jav. 3	A. 1
Castanospermum australe Cunn., Australian Chestnut	acr. 1 (0)	A. 1
Casuarina sp., Beefwood	jav. 3	B. 2
Celosia argentea L. var. *cristata* (L.) Kuntze, Cockscomb	ar. 1, hap. 4 (2), jav. 6	ar. A. 1, hap. B. 3, jav. A. 1
Celosia trigyna L., Silver Spinach	acr. 6. jav. 6	acr. C. 2, jav. E. 9
Cenchrus ciliaris L., Rhodesian Foxtail	jav. 1	E. 1, exp. infs.
Centaurea cyanus L., Cornflower	hap. 5, jav. 6	B. 2
Chenopodium album L., Goosefoot	ar. 4 (3)	B. 1
Chenopodium album L. or *opulifolium* Schrad. ex Koch, Goosefoot	hap. 4, jav. 4	A. 1
Chloris gayana Kunth, Rhodes Grass, var. Giant	acr. 1 (0), jav. 3	acr. B. 1, jav. E. 2
Id. var. Katambora	jav. 1	E. 1, exp. infs.
Chloris virgata Sw.	jav. 3	A. 1
Chrysanthemum coronarium L., Annual Yellow Daisy	acr. 6	B. 1
Chrysanthemum maximum Raymond	hap. 3	B. 1
Chrysanthemum morifolium Ramat, Florists' Chrysanthemum	acr. 1, hap. 3, jav. 3	B. 1
Cichorium intybus L., Chicory	jav. 1	A. 1
Cleome monophylla L., Spindlepod	ar. 3, jav. 5	ar. B. 1, jav. C. 4
Cleome spinosa L., Spider Flower	jav. 4	B. 1
Clitoria ternatea L.	jav. 6 (3)	B. 1
Coleus pentheri Guerke	hap. 2	B. 1
Commelina aspera Benth.	jav. 1	A. 1
Commelina benghalensis L., Wandering Jew	jav. 3 (2)	B. 4
Commelina rhodesica T. Norlindh	acr. 4, jav. 4	A. 1
Commelina sphaerosperma C. B. Cl.	acr. 4	A. 1

TABLE I (*continued*). See legend on page 337

Commelina subulata Roth.	jav. 3	A. 1
Commelina sp.	jav. 3	A. 1
Conyza aegyptica (L.) Ait.	jav. 3 (1)	A. 1
Conyza pinnata (L.f.) Kuntze	hap. 4	B. 1
Corrigiola litoralis L., Strapwort	jav. 1	A. 1
Crassocephalum sarcobasis (DC.) S. Moore	acr. 3, hap. 5 (2)	acr. A. 1, hap. B. 1
Crassocephalum sp.	jav. 4	B. 2
Crassula arborescens Willd.	jav. 4	C. 1
Crassula sp.	acr. 5 (3)	A. 1
Crotalaria juncea L., Sunhemp	acr. 4, hap. 6 (2), jav. 4	acr. B. 1, hap. B. 1, jav. E. 3
Crotalaria ochroleuca G. Don	ar. 3, hap. 5 (0)	B. 1, exp. infs.
Crotalaria spectabilis Roth., Showy Crotalaria, Early & Late	jav. 1 (0)	E. 1, exp. infs.
Cucumis myriocarpus Naud.	jav. 4 (1)	A. 1
Cucumis sativus L., Cucumber	jav. 3	B. 2
Id. var. Burpee Hybrid	jav. 6 (4)	E. 1
Id. var. English or Green Long	acr. 6 (1), jav. 6 (4)	acr. A. 1, jav. E. 1
Id. var. Palmetto	jav. 6 (4)	E. 1
Cucurbita pepo L. var. Pumpkin	acr. 6 (4), jav. 4	acr. A. 1, jav. B. 3
Id. var. Boer	acr. 6, jav. 6	E. 1
Cucurbita pepo L. var. *medullosa* Alef., Marrow, var. White Bush	jav. 5	B. 2
Cucurbita moschata Duch., Squash, var. Little Gem	jav. 6 (4)	E. 1
Id. var. Green Hubbard	acr. 6	B. 2
Cyclamen sp.	acr. 4	E. 1
Cynodon plectostachyus (K. Schum.) Pilser, Star Grass	jav. 3	E. 2, exp. infs.
Cyperus amabilis Vahl, Sedge	acr. 2, jav. 3	B. 1
Cyperus esculentus L., Water Grass	ar. 1, jav. 4	B. 2
Cyperus rotundus L., Water Grass	acr. 2, jav. 2	acr. B. 1, jav. B. 2
Cyphomandra betacea (Cav.) Sendt., Tree Tomato	acr. 5, ar. 5, hap. 4, jav. 6	acr. B. 1, ar. B. 1, hap. B. 1, jav. E. 5
Dahlia pinnata Cav.	acr. 4, hap. 3, jav. 6	acr. B. 1, hap. B. 1, jav. E. 7
Datura stramonium L., Stinkblaar	ar. 3 (1), hap. 2, jav. 5 (2)	ar. B. 1, hap. B. 1, jav. C. 1
Daucus carota L., Carrot	acr. 5 (4), hap. 4, jav. 6	acr. C. 3, hap. C. 3, jav. E. 24
Id. var. Chantenay	acr. 6, hap. 6, jav. 6	acr. D. 1, hap. D. 1, jav. E. 5
Delphinium elatum L., Hort. hybrids, Larkspur, var. Giant Pacific	ar. 4 (3), jav. 4	B. 2
Delphinium sp., Larkspur	ar. 3, jav. 5	ar. B. 1, jav. C. 2
Dianthus caryophyllus L., Carnation	ar. 3 (0), jav. 6 (5)	ar. A. 1, jav. E. 11
Diervilla sp.	jav. 3	B. 1
Digitalis purpurea L., Foxglove	ar. 3 (1)	B. 1
Digitaria ternata (Hochst.) Stapf.	ar. 2, jav. 2	B. 2
Digitaria velatina (Forsk) Beauv.	ar. 1, jav. 1	A. 1
Dimorphotheca pluvialis (L.) Moend	jav. 6	B. 1

TABLE I (*continued*). See legend on page 337

Dioscorea sp., Yam	jav. 4	A. 1
Echeveria sp.	ar. 5	E. 1
Echinochloa colonum (L.) Link, Jungle Rice	jav. 2	B. 1
Echinopsis eyriesii Zucc.	jav. 3	B. 1
Eleusine africana Kennedy O'Byrne, Rapoko Grass	acr. 3, ar. 2, jav. 3	acr. B. 1, ar. B. 1, jav. E. 5
Eleusine indica (L.) Gaertn., Rapoko Grass	acr. 3, ar. 2, jav. 3	acr. B. 1, ar. B. 1, jav. E. 5
Eragrostis arenicola C. E. Hubbard, Sandy Love Grass	acr. 2	B. 1
Eragrostis aspera (Jacq.) Nees, Rough Love Grass	acr. 3, jav. 2	B. 1
Eragrostis curvula (Schrad.) Nees, Weeping Love Grass, var. Ermelo	jav. 1	A. 1, exp. inf.
Eragrostis gummiflua Nees	jav. 3	A. 1
Eragrostis racemosa (Thunb.) Steud.	jav. 1	A. 1
Eragrostis tef (Zucc.) Trotter, Teff Grass	jav. 4	E. 2
Eragrostris viscosa (Retz) Trin., Viscid Love Grass	acr. 1, jav. 1	acr. B. 1, jav. A. 1
Erigeron floribundus (H.B.K.) Sch. Bip., Fleabane	hap. 3 (2), jav. 3	hap. C. 2, jav. A. 1
Erlangea laxa S. Moore	jav. 4	B. 2
Eschscholtzia californica Cham., Californian Poppy	jav. 5 (3)	B. 1
Euphorbia peplus L., Petty Spurge	acr. 4	B. 1
Felicia sp.	jav. 4	A. 1
Festuca arundinacea Shreb., Tall Fescue	jav. 2	E. 1
Ficus carica L. Fig	acr. 4, ar. 3	A. 1
Id., var. Adam	jav. 3	A. 1
Fimbristylis hispidula (Vahl) Kunth, Sedge	acr. 5, jav. 4 (2)	acr. B. 1, jav. D. 3
Forsythia X intermedia Zabel var. *spectabilis* (Koehne) Spaeth	jav. 3	B. 1
Fragaria X ananassa Duchesne, Strawberry, Cultivated hybrids	hap. 4	E. 6
Fuchsia hybrida vars., var. Glorious	hap. 3	A. 2
Galinsoga parviflora Cav., Quickweed	acr. 4, ar. 4, hap. 4, jav. 2	acr. E. 4, ar. C. 3, hap. E. 8, jav. E. 6
Geranium sp.	ar. 2	A. 1
Gerbera jamesonii Bolus, Barberton Daisy	acr. 5, hap. 2, jav. 5	acr. A. 1, hap. B. 2, jav. B. 3
Gisekia pharnaceoides L.	jav. 4	B. 2
Gladiolus psittacinus Hook., Parrot's Beak Gladiolus	jav. 3 (1)	A. 1
Glycine soja (L.) Sieb. & Zucc., Soya Bean, var. S.E.S. 6	jav. 6	E. 1, exp. infs.
Gnaphalium luteo-album L.	acr. 3	B. 1
Gomphocarpus fruticosus (L.) Ait. f.	jav. 6 (4)	B. 2
Gossypium hirsutum L., Upland Cotton, var. 9. L. 34	jav. 2	E. 1, exp. infs.

TABLE I (*continued*). See legend on page 337

Helianthus annuus L., Sunflower	acr. 4, ar. 5, hap. 2, jav. 5	acr. E. 2, ar. B. 1, hap. B. 2, jav. C. 6
Id. var. Dwarf Southern Cross	jav. 4	D. 1
Helichrysum monstrosum Hort., Straw-flower	jav. 4	B. 1
Hemizygia bracteosa (Benth.) Briq., Purple Top	jav. 1	A. 1
Hibiscus cannabinus L., Kenaf	acr. 6, jav. 6	E. 3
Id. var. Cuban	ar. 3 (2), jav. 5 (3)	ar. E. 1, jav. E. 2
Id. var. Egyptian	jav. 5	E. 2
Id. var. Green Java	jav. 5	E. 1
Id. var. Red Java	jav. 5	E. 1
Id. var. San Salvador	jav. 6 (4)	E. 1
Hibiscus cannabinus L., Uncultivated, Deccan Hemp	ar. 4, jav. 6	ar. B. 1, jav. E. 11
Hibiscus esculentus L., Okra, Gumbo or Lady's Finger	jav. 4	B. 1
Hibiscus rosa-sinensis L., Hibiscus Hedge	acr. 5, jav. 3	acr. B. 3, jav. C. 3
Hibiscus sabdariffa L., Rosella	ar. 1, jav. 6	ar. A. 1, jav. C. 4
Hibiscus trionum L.	jav. 6	E. 1
Holmskjoeldia sp.	jav. 5 (1)	A. 1
Hunnemannia fumariaefolia Sweet, Santa Barbara Poppy	jav. 4	B. 1
Impatiens sultanii Hook. f.	ar. 5, jav. 5	ar. E. 1, jav. C. 2
Ipomoea arachnosperma Wdw.	ar. 3, jav. 5 (3)	A. 1
Ipomoea batatas (L.) Lam., Sweet Potato	acr. 6, jav. 1	acr. C. 1, jav. A. 1
Ipomoea wightii Choisy	ar. 3, jav. 4	A. 1
Iris germanica L. hybrids, Perennial Iris	hap. 3 (2), jav. 2 (1)	hap. B. 2, jav. A. 1
Id. var. Rosy Wings	jav. 2	A. 1
Kniphofia sp., Red Hot Poker	acr. 1	B. 1
Lablab niger Medik., Dolichos Bean, var. Brown Seeded	jav. 4	B. 1
Lactuca sativa L., Lettuce	acr. 3, hap. 4, jav. 3 (1)	B. 2
Lathyrus odoratus L., Sweet Pea	jav. 4	E. 3
Leucas martinicensis R. Br., Bobbin Weed	hap. 3, jav. 3	hap. E. 2, jav. E. 1
Leucas sp.	hap. 3	A. 1
Lightfootia marginata A.D.C.	jav. 1	A. 1
Ligustrum lucidum Ait., Privet Hedge	jav. 6	E. 1
Linum sp., Flax	ar. 4 (1)	A. 1
Lippia javanica (Burm. f.) Spreng.	jav. 1	A. 1
Lobelia erinus L.	hap. 1, jav. 5	A. 1
Lolium multiflorum Lam., Italian Rye Grass	jav. 2	E. 1
Lolium perenne L. X *Lolium multiflorum* Lam., Rye Grass, N. Z. Hybrid 1	jav. 6 (4)	C. 1
Lupinus albus L., Field Lupin, var. Bitter	jav. 2 (1)	B. 1
Id. var. Sweet Weiko	ar. 3, jav. 2	B. 1
Lupinus regalis Bergmans, Garden Lupin	jav. 2	B. 2
Lycopersicon esculentum Mill., Tomato	acr. 6, ar. 3, hap. 2, jav. 6	acr. E. 10, ar. B. 1, hap. E. 4, jav. E. 54
Id. var. Beauty	jav. 5	E. 1

TABLE I (*continued*). See legend on page 337

Id. var. Essex Wonder	hap. 6	E. 1
Id. var. Marglobe	acr. 6 (4), ar. 6 (3), hap. 6, jav. 6	B. 1
Id. var. Marvel	ar. 3, hap. 4, jav. 4 (2)	E. 2
Id. var. Pendrosa X Oxhart	jav. 6	E. 1
Id. var. Stoner's Money Maker	hap. 6	E. 1
Lycopersicon esculentum (L.) Mill. ex var. *cerasiformis* Alef., Cherry Tomato	jav. 6	B. 2
Lycopersicon peruvianum Mill.	acr. 1, jav. 2	B. 2, exp. infs.
Mariscus hemisphaericus (Boeck.) C.B. Cl.	jav. 3 (0)	A. 1
Medicago lupulina L., Yellow Trefoil	hap. 4	E. 1
Medicago sativa L., Lucerne or Alfalfa	acr. 1, jav. 5	acr. A. 1, jav. E. 4
Id. var. Buffalo	jav. 5 (0)	E. 1, young plants
Id. var. Hunter's River	jav. 4	E. 1
Id. var. Washington	jav. 2	E. 1
Mentha spicata L., Garden Mint	hap. 2	E. 3
Morus sp., Mulberry	jav. 4 (1)	A. 1
Musa nana Lour. Banana, var. Dwarf Cavendish	acr. 3, ar. 3, jav. 4	acr. B. 2, ar. A. 1, jav. E. 25
Musa paradisiaca L., Plantain	jav. 4	C. 4
Musa sapientum L., Banana, var. Lady Fingers	jav. 3	B. 2
Nicandra physalodes (L.) Gaertn., Apple of Peru or Chinese Lantern	ar. 3, hap. 2, jav. 2	ar. B. 1, hap. A. 1, jav. C. 4
Nicotiana tabacum L., Fire Cured Tobacco, var. Western	acr. 6, jav.6	E. 1
Nicotiana tabacum L., Turkish Tobacco	jav. 3	B. 4
Id. Type Samsum, var. Baffra	jav. 4	C. 1
Id. var. Janik	jav. 4	C. 1
Id. Type Selouk	jav. 5	E. 1
Nicotania tabacum L., Virginia tobacco	acr. 6, jav. 6	acr. E. 4, jav. E. 120
Id. var. Bonanza	jav. 6	E. 8
Id. var. Delcrest	jav. 6	E. 10
Id. var. Hicks	acr. 1, ar. 6 (2), hap. 4, jav. 6	acr. E. 1, ar. B. 1, hap. B. 2, jav. E. 30
Id. var. Gold Dollar	jav. 6	E. 2
Id. var. Jamaica Wrapper	jav. 6	E. 7
Id. var. K 401	jav. 6	E. 7
Id. var. White Stem Orinoco	acr. 6, hap. 4, jav. 6	acr. E. 3, hap. A. 1, jav. E. 20
Id. var. Yellow Mammoth	jav. 6	E. 3
Nierembergia hippomanica Miers	jav. 6	E. 3
Oenothera sp., Evening Primrose	hap. 1	A. 1
Oldenlandia caespitosa Hiern	hap. 1	A. 1
Oldenlandia herbacea (L.) Roxb.	acr. 5, jav. 2	acr. E. 1, jav. A. 1
Oldenlandia linearis DC.	ar. 2, jav. 2	A. 1
Oxalis corniculata L., Sorrel	jav. 5 (2)	C. 4
Oxalis latifolia H.B.K.	ar. 1	A. 1
Oxalis obliquifolia Steud ex A. Rich	jav. 3	B. 1
Oxalis semiloba Sond., Sorrel	jav. 4	B. 2

TABLE I (*continued*). See legend on page 337

Oxalis sp.	acr. 3	A. 1
Panicum coloratum L., Makarikari Grass	jav. 4	B. 1
Id., Bambatsi Panicum, var. Makarikariensis	jav. 2	B. 2
Passiflora edulis Sims, Granadilla	acr. 6, jav. 6	acr. B. 1, jav. C. 6
Pastinaca sativa L., Parsnip	acr. 3, hap. 6, jav. 5	acr. A. 1, hap. E. 2, jav. E. 8
Id. var. Guernsey	jav. 4	E. 1
Pavonia columella Cav.	acr. 2, jav. 5	acr. B. 1, jav. B. 2
Pelargonium zonale Ait.	hap. 3, jav. 3 (1)	hap. B. 2, jav. A. 1
Pennisetum purpureum Schumach., Napier Fodder or Elephant Grass	jav. 1	C. 1, exp. infs.
Pennisetum typhoides (Burm.) Stapf. & Hubbard, Maize or Pearl Millet	jav. 2	E. 4
Penstemon grandiflorus Nutt.	ar. 4 (2), jav. 4 (2)	A. 1
Pentanisia sykesii Hutch. subsp. otomeriodes Verdi	jav. 3	B. 1
Petrea volubilis Jacq., Queen's Wreath	jav. 4 (1)	B. 2
Petroselinum crispum (Mill.) Airy-Shaw, Parsley	acr. 6, hap. 4, jav. 6	acr. B. 1, hap. A. 1, jav. D. 9
Petunia hybrida Vilm.	acr. 6, hap. 3, jav. 6	acr. B. 1, hap. B. 2, jav. B. 2
Phaseolus limensis Macf., Butter Bean	jav. 4	B. 2
Phaseolus lunatus L., Lima Bean	jav. 6	C. 4
Id., White Sugar Bean	acr. 5, jav. 6	acr. B. 1, jav. A. 1
Phaseolus multiflorus Willd., Runner Bean	ar. 3, jav. 5	ar. A. 1, jav. C. 4
Id. var. Everbearing	acr. 6, jav. 4	B. 1
Id. var. Kentucky Wonder	jav. 6	C. 2
Id. var. Westralia	jav. 6	B. 1
Phaseolus vulgaris L., Dwarf Bean	acr. 6 (3), ar. 5, hap. 2, jav. 6	acr. C. 2, ar. B. 2, hap. B. 2, jav. E. 15
Id. var. Burpee Stringless	jav. 6 (4)	A. 1
Id. var. Long Tom	jav. 6	C. 2
Id. var. Refugee	jav. 6	C. 1
Id. var. Stringless	jav. 4 (3)	B. 1
Id. var. Tender Green	jav. 5	A. 1
Id. var. Top Crop	jav. 4	A. 1
Phlox drummondii Hook.	jav. 3	B. 1
Physalis peruviana L., Cape Gooseberry	hap. 4, jav. 3	hap. B. 2, jav. B. 3
Pisum sativum L., Garden Pea	jav. 6, ar. 4	ar. B. 1, jav. E. 15
Id. var. Burpeana	jav. 4	B. 1.
Id. var. Clipper	jav. 4	B. 1.
Id. var. Greenfeast	acr. 6, hap. 3 (2), jav. 6	acr. C. 1, hap. E. 3, jav. E. 6
Id. var. Onward	ar. 3	B. 1.
Id. var. Progress Type	acr. 4	A. 1
Id. var. Stratagem	jav. 2	B. 1.
Podranea brycei Sprague, Zimbabwe Creeper	jav. 4	B. 1.
Polianthes tuberosa L., Tuberose	jav. 3	A. 1
Polycarpaea corymbosa (L.) Lam.	jav. 2	A. 1

TABLE I (*continued*). See legend on page 337

Portulaca oleracea L.	acr. 3, hap. 2	A. 1
Prunus persica (L.) Batsch, Peach	jav. 3 (1)	B. 2
Id. var. Boland	jav. 2	A. 1
Id. var. Elberta	acr. 6	A. 1
Id. var. Killie krankie	jav. 4	B. 1
Pseudarthria hookeri Wight & Arn.	hap. 2	A. 1
Raphanus sativus L., Raddish, var. Sparkler	acr. 1, jav. 3	acr. A. 1, jav. B. 2
Rehmannia angulata Hemsl.	ar. 4 (2), jav. 4	ar. B. 1, jav. C 2
Rheum rhaponticum L., Rhubarb	ar. 4, jav. 2	ar. B. 2, jav. A. 1
Rhynchelytrum repens (Willd.) C. E. Hubbard, Natal Red Top	jav. 4 (3)	B. 2
Richardia brasiliensis (Moq.) Gomez, Mexican Clover	jav. 2	B. 1
Ricinus communis L., Castor Oil Plant, Uncultivated	ar. 3	B. 1
Id. var. Mauther's Spineless Dwarf	jav. 1	A. 1. exp. infs
Rosa odorata Sweet, Rose	hap. 4 (0)	A. 1
Rottboellia exaltata L.f., Guinea Fowl or Kokomo Grass	acr. 2	B. 1
Rubus sp.	jav. 4 (0)	A. 1
Rumex angiocarpus Murb.	hap. 4 (3)	C. 3
Salix caprea L., Pussy Willow	jav. 4	B. 1
Salpiglossis sinuata Ruiz & Pav.	jav. 4	B. 1
Salpiglossis sp.	hap. 4	B. 1
Salvia officinalis L., Sage	jav. 3	B. 1
Salvia patens Cav.	acr. 2, jav. 5	acr. B. 1, jav. B. 2
Salvia sp.	jav. 1	A. 1
Saponaria officinalis L. flore pleno, Soapwort	jav. 5 (2)	A. 1
Schizanthus sp. Poor Man's Orchid	jav. 1 (0)	A. 1
Senecio cineraria DC., Dusty Miller	hap. 4, jav. 4	C. 3
Senecio discifolius Oliv.	jav. 4	B. 1
Senecio latifolius DC. S. l., Rhodesian Ragwort	hap. 3 (0)	B. 1
Sesbania microphylla Harms.	jav. 6	B. 1
Setaria homonyma (Steud.) Chiov., Fan Leaf Timothy Grass	jav. 1	A. 1
Setaria lindbergiana (Nees) Stapf., Rhodesian Tussock Grass	acr. 3, jav. 1	A. 1
Setaria pallide-fusca (Schumach.) Stapf. & Hubbard, Annual Timothy Grass	ar. 3, jav. 4 (3)	ar. A. 1, jav. B. 2
Setaria sphacelata (Schumach.) Stapf. & Hubbard ex M. B. Moss	jav. 4	B. 2
Setaria verticullata (L.) Beauw., Cat's Tail	jav. 2 (1)	A. 1
Setaria sp., Ngamiland Grass	jav. 2	C. 1. exp. infs.
Silene nutans L.	jav. 4 (2)	A. 1
Solanum capsicastrum Link, False Jerusalem Cherry	acr. 4 (2), ar. 4, hap. 4, jav. 6	acr. ar. & hap. B. 1, jav. E. 2
Solanum indicum L.	jav. 3	B. 1
Solanum melongena L., Eggfruit Plant	ar. 2, hap. 2, jav. 6	ar. & hap. A. 1, jav. E. 17

TABLE II

Known numbers of plant species found harbouring identifiable Meloidogyne females together with the numbers of infested sites.

	Meloidogyne species			
	incognita var. acrita	*arenaria*	*hapla*	*javanica*
Host plants	84	54	71	219
Infested sites	46	27	56	347

The author wishes to express his thanks to Mr. A. L. Taylor and Dr. V. H. Dropkin for some initial assistance in the confirmation of root-knot nematode identifications; Dr. H. Wild, Miss K. Sturgeon, Mr. R. B. Drummond and Mr. J. B. Phipps of the Branch of Plant Pathology and Botany for plant identifications and nomenclature and to Mr. J. A. Whellan and Dr. C. E. Taylor for helpful advice and proof correction. Thanks are also due to Mr. A. Angus and Mr. J. Hadfield for the supply of much of the material from Northern Rhodesia.

ZUSAMMENFASSUNG

Meloidogyne javanica, M. incognita var. *acrita, M. hapla* und *M. arenaria* sind bisher in Rhodesien und Nyasaland nachgewiesen worden. Mehrere tausend Untersuchungen, meistens natürliche Infektionen, wurden zu Wirtslisten zusammengestellt, die hauptsächlich landwirtschaftliche Gewächse von wirtschaftlicher Bedeutung, Gemüse, Gartenblumen und Unkräuter von Ackerboden umfassen. Berichtet wird über den Grad der durch Nematoden verursachten Gallen und Eimassen sowie über die Verbreitung der *Meloidogyne*-Arten. Es wird die Ansicht vertreten, dass *M. javanica* einheimisch sein kann, während die übrigen drei Arten des Wurzelgallennematoden möglicherweise mit Pflanzenmaterial eingeschleppt wurden. Die Rasse von *M. javanica,* die *Arachis hypogaea* und *Gossypium hirsutum* befällt, unterscheidet sich in dieser Hinsicht von der Rasse, die SASSER (1954) aus Maryland, U.S.A. beschreibt.

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Received for publication: April 29, 1958.

BOOK REVIEW

ANDRÁSSY, I. *Szabadonélö fonálférgek — Nematoda libera* in: SZÉKESSY: *Fauna Hungariae,* Lief. 36. Budapest, 1958. pp. 362, Akadémiai Kiadó.

The enormous progress, or let us say, the coming into its own, of soil biological researches in the past ten years has resulted in an unexpected advance in the more complete understanding of several microscopical animal groups living in the soil, namely certain Protozoa, Rotatoria, Nematoda, Collembola, Acarina. To stress my point, I give but one example taken from the free-living Nematodes. Whilst, during the 10-15 years before the end of World War II, there were but five of us in Europe working on land and fresh-water Nematodes, there are now many dozens of workers in this field. Inevitably the question arises of what comprehensive works are at the disposal of beginners in Nematology. Aside from the excellent and modern, general comprehensive works of CHITWOOD and GOODEY, the situation is far less favourable as regards identification manuals. In spite of the fact that in the past few years better and better genus and family monographs have been published, research workers, when investigating the land or fresh-water Nematodes of a given European area, are still compelled to have recourse to the books of MICOLETZKY 1921 and W. SCHNEIDER 1939. Both works were first-class references in their time, indeed, they are still basically sound and effectively usable. However, two important things have happened in the 37 years since the publication of MICOLETZKY's book. Firstly, a whole host of new species, genera, and even families have been described in the past decades; secondly, the systematics of free-living nematodes has basically altered, and in the delimiting of species certain new specific characters have been found which were not — indeed, could not have been — taken into consideration by the earlier authors and taxonomists.

These considerations prompted me to review or to call attention to I. ANDRÁSSY's book in the series of the new Fauna Hungariae, published by the Hungarian Academy of Sciences. It could justly be said that, even if my previous remarks are sound, what could one do with a book published in the Hungarian language? The question is wholly justified. The following facts, however, necessitate the review of the book in spite of its Hungarian idiom.

This work, though published in the series Fauna Hungariae can, knowing the potential range of the free-living nematodes, be used not only in Hungary but in every other part of Europe, in a similar way as SCHNEIDER's book in the series „Die Tierwelt Deutschlands". ANDRÁSSY's work supplies the keys for the identification of 507 species, and embraces the results of the most modern systematical researches, so that the book includes three-fourths of the species known from Europe up to now. Apart from the text, its highest value for every nematologist lies in its 700 figures. About 98 per cent of the figures are the original drawings of the author. This must be emphasized for the very good reason that, in this work, we do not come up against the same old and familiar drawings published and re-published from book to book, but find original drawings, revealing also the novel features of identification even of species described in the last century. By leafing through the pages of the book, one also notices the groups of genera still in need of revision. I sincerely hope that, on the basis of the results of recent intensive research work, we will, in a few years time, be in the position to have a modern reference work or monograph of the European fauna in a Congress language.

Dr. Á. Soós
Budapest.

N.B. Subscriptions for the book should be sent to its distributors: Meulenhoff & Co. N.V. — Amsterdam; or "Kultura", Budapest 62, póstafiók 149.

SOCIETY OF EUROPEAN NEMATOLOGISTS

V International Nematology Symposium at Uppsala (Sweden), August 1959.

In accordance with the resolution of the IV International Nematology Symposium held in Hamburg in September 1957, the next Symposium will take place at Uppsala, August 10th-13th 1959.

The first three days will be devoted to lectures and discussions on plant nematodes and the diseases they cause. On the fourth day an excursion will be arranged to Ultuna (Branch Station of Swedish Seed Association) and to Stockholm (State Plant Protection Institute).

If possible, there will also be a two-day excursion to Hälsingland to see nematode infestations in the field.

After the Symposium a post-symposium will be held in Åkarp (near Malmö) at the Substation of the State Plant Protection Institute.

Accommodation

During the symposium participants will have the choice of staying in private homes (student chambers of good standard costing up to 8 sw. crowns (16/—) a day), or hotel rooms (costing from about 13 sw. crowns (26/—) upwards a day). The conference fee will probably not exceed 10 sw. crowns (20/—). More definite information will be sent later.

Preliminary announcements and enquiries concerning participation in the Symposium are to be sent to *O. Ahlberg, Head of Zoology Department, State Plant Protection Institute, Stockholm 19, Sweden,* as soon as possible.

O. Ahlberg, President
K. Lindhardt, Secretary

INDEX OF VOLUME III

Anguillulina obtusa, taxonomy 136.
Anguina tritici, effects on wheat and barley varieties 24.
Aphelenchoides dactylocercus n.sp., morphology 229.
sacchari n.sp., morphology 231.
ritzema-bosi and *Corynebacterium fascians*, 244.
Diplogaster lepidus n.sp., morphology 295; key to species 298.
Ditylenchus dipsaci, effects on wheat and barley varieties 24; in narcissus 73.
myceliophagus n.sp., morphology 91.
Dolichodorinae, status 301.
Gottholdsteineria n.g. 53.
Helicotylenchus, genus description 52.
Hemicycliophora pauciannulata n.sp., morphology 17.
oostenbrinki n.sp., morphology 18.
paradoxa n.sp., morphology 21.
typica, male spear 140.
Heterodera, larval emergence from cysts 236.
goettingiana, in eastern England 257.
humuli, morphology, biology 269.
major, population studies 274.
rostochiensis, root diffusate 6, 108, 149, 173; day length and cyst formation 81; hatching factor 154; development in sterile cultures 308.
schachtii, resistance 127; root diffusate of *Brassica rapa oleifera* DC. 315.
Hoplolaiminae revision 44; key to the genera 51.
Hoplolaimus, genus description 54.
gracilidens n.sp., morphology 98.
uniformis, synonymy with *Rotylenchus robustus* 42.
tylenchiformis, redescription, synonymy with *H. coronatus* 44.
Macrotrophurus arbusticola n.g. n.sp., 301.
Meloidogyne spp., in Rhodesia and Nyasaland 333; chemical analysis of cuticle and egg sac 205.
Nematoda, in mushroom compost 91; influence of osmotic concentration on hatching 115; screening antibiotics 143; production of cellulase and chitinase 179; secondary action of DD 213; cauliflower disease 244
Paraphelenchus myceliophthorus n.sp., morphology 1.
Pratylenchoides crenicauda n.g. n.sp., morphology 136.
Pratylenchus penetrans, inoculation in potatoes 31.
Radopholus inaequalis n.sp., morphology 100.
neosimilis n.sp., morphology 103.
similis, morphological variation 184.
Rotylenchoides brevis n.g. n.sp., 328.
Rotylenchoidinae n. subfamily 328.
Rotylenchus, genus description 51.
goodeyi nom. nov., for *R. robustus* auctt. nec de Man, 42.
robustus, synonymy with *H. uniformis* 42.
obtusus, taxonomic position 136.
Scutellonema n.g., 54.
Sphaeronema minutissimum n. sp., morphology 169.
Tylenchulus semipenetrans, life history 283.
Tylenchus robustus, taxonomic position 34.
Xiphinema, key to species 59.
hallei n. sp. 60.
yapoense n.sp. 62.
diversicaudatum, morphology 64.
ebriense n.sp. 65.
campinense, morphology 68.
parasetariae n.sp. 71.
setariae n.sp. 69.